SURVEY OF CANADIAN NATIVE PLANT SPECIES FOR RESISTANCE TO SALT AND METAL STRESS

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Environment and Sustainability

In the School of Environment and Sustainability

University of Saskatchewan

Saskatoon

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ABSTRACT

There are over 21, 000 federally managed metal contaminated soil sites in Canada, not including sites managed by the private sector or sites affected by other contaminants requiring some level of remediation. One of the most economical remediation options, especially on a large scale, is phytoremediation. Phytoremediation is a process where plants and their associated microbes uptake, break down, or immobilize target soil contaminants. Native plant species are important to consider for phytoremediation since their lifecycles are acclimatized to local soil and weather and are not at risk of becoming invasive. The purpose of this thesis was to assess Canadian native plant species used in soil reclamation for their resistance to metals and salts found in oil sands mine tailings. To determine germination and growth inhibition concentrations of salts and metals, seeds were exposed to various metal and salt concentrations in semi-solid water agar. Germination and early growth of *Achillea millefolium* (common yarrow), *Astragalus canadensis* (Canadian milkvetch), *Calamovilfa longifolia* (Prairie sandreed), *Koeleria macrantha* (Prairie Junegrass), and *Vicia americana* (American vetch) were assessed in a lab bioassay using semi-solid water agar, and in a greenhouse experiment, using soil. The semi-solid water agar and soil were artificially contaminated with either a metal [$Cd(NO_3)_2$, $Cr(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$], or salt (KCl, NaCl, K_2SO_4, Na_2SO_4).

In the bioassay, germination was inhibited by salts (0, 1000, 10 000, 100 000 mg kg^{-1}) for all species at concentrations exceeding 1000 mg kg^{-1}. *Koeleria macrantha* was the only species that was inhibited by metals at 20 and 50 mg kg^{-1} Cd and Cu. Roots and shoots exhibited stunting for many of the metal concentrations, and in salt concentrations exceeding 1000 mg kg^{-1}.

In the greenhouse experiment, metal concentrations (0, 10, 20, 50 mg kg^{-1}) had no effect on germination and there was no significant difference ($p<0.05$) between the biomass of control plants and plants grown in the metal contaminated soil. Seeds struggled to germinate in salt contaminated soil (0, 1000, 5000 mg kg^{-1}), mostly in the chloride amended soil, and produced very little biomass. The most promising candidate from the experiments was *A. millefolium* since it had the highest germination, and the longest root and shoot lengths in the bioassay study. Also, it produced the highest biomass in the greenhouse study, and was able to grow in chloride amended soil, which significantly inhibited other species. Results suggest *A. millefolium* should be the focus of future studies.

ACKNOWLEDGEMENTS

This research was made possible through funding by the National Sciences and Engineering Research Council of Canada for the Forest Watershed and Riparian Disturbance (FORWARD III) Project, and through the Harvey Graduate Scholarship.

A very special thank you to my supervisor, Dr. Jim Germida, for his patience, help, and support throughout this process. Thank you to my committee members, Drs. Markus Hecker, Ken Van Rees and Fran Walley, for their thoughtful questions and insights. I would also like to thank my external examiner, Dr. Karen Tanino, for her great suggestions and comments.

Thank you to Drs. Diane Knight and Peiqiang Yu for their stats know-how, and to Drs. Jeff Schoenau and Ryan Hangs for supplying me with soil for my experiments. Thank you to the lab members of 5E25 for all of your help and support, especially Eduardo, Jorge, Natalie, and Panchali.

I would like to extend my deepest gratitude to faculty and staff in the Soil Science Department and in the School of Environment and Sustainability. It has been truly wonderful getting to know you these past few years. A very special thank you to Cathy, I would not have been able to do this without you.

Finally, I would like to thank my family and friends, both here and back home, for their encouragement and moral support. To Leo, thank you for everything. I am looking forward to our next adventure. Pío pío pío

DEDICATION

For the most compassionate, thoughtful, and strongest person I know, my mom.

Thank you for your continual support and wise words, I wouldn't be who I am today without you. Love you!

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
As	arsenic
Ca	calcium
Cd	cadmium
Co	cobalt
Cr	chromium
Cu	copper
EC	electrical conductivity
EDTA	ethylenediamine tetraacetic acid
Fe	iron
GDP	gross domestic product
Hg	mercury
HSD	honest significant difference
ICP-OES	inductively coupled plasma optical emission spectrometry
K_2SO_4	potassium sulfate
KCl	potassium chloride
Mn	manganese
Na	sodium
Na_2SO_4	sodium sulfate
NaCl	sodium chloride
Ni	nickel
Pb	lead
PEG	polyethylene glycol
R:S	root:shoot ratio
Se	selenium
TSA	tryptic soy agar
Zn	zinc

1.0 INTRODUCTION

After Saudi Arabia and Venezuela, Canada is home to one of the largest oil deposits in the world (Government of Alberta, 2017a). Mineral mining is also an important pillar of the Canadian economy (Natural Resources Canada, 2017). The Canadian mining and oil sectors accounted for approximately 8 % of the country's gross domestic product (GDP) in 2016 (Statistics Canada, 2017).

Bitumen is an unconventional oil source derived from oil sands which are made up of sand, clay, water and bitumen (Government of Alberta, 2017b). The process of refining and upgrading bitumen is water intensive requiring various chemicals and heat to release the thick bitumen from the sand. The leftover water-sand slurry is pumped into tailings ponds where the solids settle out and the water is reused (Canadian Association of Petroleum Producers, 2017). During the refining and upgrading processes, impurities found in the bitumen, such as metals and salts, are released and concentrated in the tailings ponds (Government of Alberta, 2015). Some of the most prevalent metals and salts found in the oil sand mine tailings are sulfates, chlorides, cadmium, copper, chromium and nickel (Renault et al., 1998; Allen, 2008a; Mahdavi et al., 2013). Excess metals and salts can impede plant growth, and negatively impact wildlife and soil biota through direct contamination (Foy et al., 1978) or indirectly by changing soil pH causing nutrient imbalances (McLean, 1973).

Tailings ponds are eventually retired and reclaimed. According to Alberta legislation, the large tailings area must be returned to an ecosystem productivity state equal or greater to its pre-mined state (Government of Alberta, 2013; Province of Alberta, 2016). Much of the sought after bituminous sand is found in the boreal forest of northern Alberta. The reclaimed area is not required to be identical to its previous state, rather it must be representative of the boreal ecosystem where the oil sands are located. Usually the overburden, organic matter and soil found on top of the minable sand, is used to cap the tailings ponds and then planted using native vegetation (Government of Alberta, 2017c). But what about the residual impurities in the mine tailings leftover from the extraction process?

There are numerous benefits to using native plant species for re-vegetation of reclaimed tailing sites. The most obvious is that these plants will grow surrounded by like vegetation, and the second is that the plants are adapted to the area's climate and are not at risk of becoming an invasive species. Many plant species native to Alberta have been approved for use in soil reclamation (Government of Alberta, 2001; Gosselin et al., 2010; Smreciu et al., 2013); however, little is known regarding their remediation potential for oil sands mining and tailing sites.

Soil remediation goes one step further than reclamation whereby the soil is decontaminated before being reclaimed. A few methods exist for soil remediation; however, these methods can be labor intensive and costly (Glick, 2010). Phytoremediation is more cost effective, less labor intensive, and preserves environmental integrity better than physical or chemical alternatives. Phytoremediation is a process where plants and their associated microbes uptake, immobilize, or breakdown target soil contaminants (Salt et al., 1995; Zhang et al., 2013). Additionally, some plants are capable of storing contaminants in their biomass effectively removing them from the soil system (Memon and Schröder, 2009).

1.1 Objectives and hypotheses

The main purpose of this research was to increase the information available on Canadian native plant species for their resistance to metals and salts found in oil sand tailings to improve remediation guidelines. The objectives of the study were to 1) determine the effects of isolated metal and salt stress on germination, and root and shoot growth of native plant species in soil-less medium, and 2) determine the effects that metals and salts have on plant biomass when grown in artificially contaminated agricultural soil. To accomplish these aims, two studies were conducted to assess the following null hypotheses:

1. H_{01}: There will be no statistical differences in final germination among plant species under controlled conditions;
2. H_{02}: There will be no statistical differences in final germination and root and shoot growth between treatment groups and the control or among treatment groups when seeds are exposed to different metal and salt concentrations in soil-less medium; and
3. H_{03}: There will be no statistical differences in seed germination or biomass produced between treatment groups and the control or among treatment groups when plants are exposed to metal or salt contaminated soil.

This thesis is presented in manuscript-style format. Following this introduction, a literature review is presented in Chapter 2, followed by two research studies (Chapters 3 and 4). Chapter 3 explores the screening of plant species native to western Canada for their germination and growth in metal or salt contaminated semi-solid medium. Chapter 4 focuses on successful plants from the first study which were planted in contaminated soil and their germination and biomass was measured. Finally, Chapter 5 draws conclusions of the main research findings and suggests future directions. Since each chapter was written as a stand-alone manuscript, there may be some information repeated among chapters.

2.0 LITERATURE REVIEW

2.1 Soil pollution

Soil contamination affects approximately 16 % of the world's total land area (EEA, 2003) stemming mostly from anthropogenic activities. Mining poses one of the highest threats to the environment because mines generate large amounts of waste, their wastes have the highest potential of containing toxic compounds compared to other anthropogenic activities, and they are found worldwide (Thornton, 1996; Chakradhar, 2004; McKenna Neuman et al., 2009; Brotons et al., 2010; Csavina et al., 2011). Mine tailings, waste disposal, and atmospheric deposition, among others, spread pollution through ground water and erosion of particulates (Pelletier, 2006). Particulate deposition by wind (dry deposition) has the largest potential for transport since it is not defined by topography the same way that water and soil are and can travel rapidly, depending on wind speed (Kolpin et al., 1998; Kersting et al., 1999; Mulligan and Yong, 2004; Braune et al., 2005). Communities living downwind have reported elevated levels of contaminants in soils caused by atmospheric particulate deposition (Barrie et al., 1992; Perry et al., 1999; Chu et al., 2003; Park et al., 2004; Zhai et al., 2008; Gallon et al., 2011). These contaminants can enter the food chain through the consumption of plants grown in affected soil and this can have negative impacts on local communities (Nawab et al., 2016). In soils affected by metals, vegetation can greatly influence their mobility both by changing the organic matter content of the soil and through the modification of redox conditions (Wang et al., 2002; Wenzel, 2009). Metals are among the main soil contaminants worldwide that require remediation (Alloway, 1990) and are mainly emitted due to anthropogenic activities (Nriagu, 1989). Using plant cover to decrease soil erosion can help mitigate the spread of pollution (Wuana and Okieimen, 2011). Both soil reclamation and remediation use this strategy.

2.2 Soil remediation options

Physical removal of contaminated soil is a labor and economically expensive practice. Soil is removed for disposal in a landfill or incinerator (Ha et al., 2009), or taken to a cleaning facility where soil is washed and treated to remove the target contaminants through a combination of physical and chemical remediation (Glick, 2003; Alkorta et al., 2004). Once clean, soil can be

returned to its original area. Disposal of soil poses its own risks since landfills can leak harmful substances, threatening groundwater (Remon et al., 2005). Physical remediation is not only costly and labor intensive, it is also damaging to the surrounding environment. Landscape changes can affect how different trophic levels interact and survive in the newly altered environment (Naeem et al., 1995; Symstad et al., 1998). For example, the soil microbial community can shift after disturbance and can take a long time to recover (Buckley and Schmidt, 2003; Jangid et al., 2010; Duchicela et al., 2012).

Chemical removal of contaminants occurs when a chemical compound is added to the soil, generating a chemical reaction which targets the contaminant(s) of interest. For example, removing metals from soil can occur when a chelating agent, such as ethylenediamine tetracetic acid (EDTA), is applied to the soil increasing metal mobility and bioavailability (Colls and Hall, 2004; Wu et al., 2010; Olaniran et al., 2013; Chirakkara et al., 2016). This increase allows metals to be more readily taken up by vegetation. However, the now mobile metal ions can also move through the soil profile into the groundwater. Salts flushed from the soil can also leach into groundwater (as summarized by Alberta Environment, 2001).

Finally, there are a numerous types of phytoremediation that can greatly reduce clean-up costs and environmental disturbance compared to physical and chemical remediation technologies (Glick, 2010). Some plants can accumulate contaminants in their tissues that can be harvested and removed. Furthermore, phytoremediation deals with the problem, rather than outsourcing or causing new waste streams (Memon and Schröder, 2009). Over time, concentrating contaminants in plant biomass from soil reduces the amount of waste requiring treatment. Chemical and physical soil remediation are not always feasible at a large scale due to the large physical and economic investments that are required (Tappero et al., 2007). Also, the cost of phytoremediation can be as low as 10 % of that of physical removal (Grommen and Verstraete, 2002) while maintaining the soil's biofertility (Batty and Dolan, 2013).

2.3 Phytoremediation

Phytoremediation is a process where plants and their associated microbes are used to degrade, uptake or immobilize target soil contaminants (Salt et al., 1995; Zhang et al., 2013). Many types of phytoremediation exist including: phytodegradation, phytoexclusion, phytoextraction, phytomining, phytostabilization, phytostimulation, phytovolatilization and rhizoremediation. Each type focuses on a different pathway of remediation (i.e., contaminant uptake vs. contaminant

storage). Microbially assisted phytoremediation (i.e., bioremediation) can induce competition between the introduced microbes and the local non-remediating populations (Gerhardt et al., 2009). If local populations outcompete the introduced microbes, bioremediation does not occur. However, if the introduced microbes outcompete the local populations there can be shifts and losses to the native soil microbiota (MacNaughton et al., 1999).

Soil phytoremediation has many advantages over other remediation options including: relatively low environmental disruption, soil structure preservation, lower economic inputs, less physically demanding, and is carbon dioxide neutral (Glick, 2003; Peuke and Rennenberg, 2005). In the United States an estimated 7-8 billion USD was spent on soil remediation, with about one third allocated to metal remediation (Bennett et al., 2003). Phytoremediating the same area would cost significantly less, about 10 %, of the estimated cost of traditional remediation options (Grommen and Verstraete, 2002). Disadvantages include: a longer time commitment (i.e., decades), upkeep requirements (i.e., replanting, harvesting, etc.), and limited contaminant remediation based on the plant's rooting zone (Cunningham and Ow, 1996).

To decrease the time required to phytoremediate an area, plants and microbes are being genetically engineered to be more efficient at contaminant uptake and breakdown. Plant examples include *Arabidopsis thaliana* (L.) Heynh. (Thale cress) and *Nicotiana tabacum* L. (tobacco) that have been modified to volatilize mercury (Hg) using organomercurial lyase (*merB*) and mercuric reductase (*merA*) (Heaton et al., 1998). Microbial examples include *Burkholderia cepacia* L.S.2.4 that was modified with a plasmid from *B. cepacia* G4 which lead to increased degradation of toluene and a decrease in phytotoxiciy (Barac et al., 2004), and *Deinococcus radiodurans* Brooks & Murray, a radiation resistant bacterium, that has been modified using *Escherichia coli* (Migula) BL308 strain to reduce Hg (II) to a less toxic Hg species (Brim et al., 2000).

2.4 Soil Contaminants

Anthropogenic activities such as mining, oil production and other industrial practices can result in large-scale soil contamination (Raskin et al., 1997; Audet and Charest, 2007; Babu et al., 2013). Soil contamination is also a human health concern since contaminants can be dispersed through dust (Barrie, 1986), or leach through the soil profile into the groundwater causing large scale disturbance (Camobreco et al., 1996). Soil contaminants can include metals, salts, and polycyclic aromatic hydrocarbons; all of which can have negative impacts on plant health (Henner et al., 1999; Allakhverdiev et al., 2000). Additionally, plants growing in metal contaminated soil

can, when ingested, cause serious health problems (Prodgers and Inskeep, 1991; Agency for Toxic Substances and Disease Registry, 2017). Many contaminants degrade over an extended time (i.e., half-life of naphthenic acid is 12.8-13.6 years) (Han et al., 2009), whereas others do not breakdown (i.e., metals).

2.4.1 Fort McMurray oil sands

In 2014, 10 % of Canada's gross domestic product (GDP) came from the mining and agriculture sectors (Government of Canada, 2015). This is concerning since both sectors are main contributors to large scale soil contamination which includes metals, salts, pesticides, naphthenic acids, and hydrocarbons. The oil reserves in Alberta alone account for approximately 5 % of Canada's 2012 GDP (IHS CERA, 2014).

Alberta's oil sand deposit is divided into three main reserves: Athabasca, Cold Lake, and Peace River (Government of Alberta, 2017a). Though an unconventional oil source, the oil sand reserves in Alberta contain approximately 166 billion barrels of oil, over an area of 142 200 km^2 (Government of Alberta, 2017a), which is about twice the size of New Brunswick, or 1.5 % of the total area of Canada. Only about 20 % of the oil in the reserves is accessible through mining; the other 80 % requires *in-situ* production, new technology or better prices to make recovery economically favorable (Government of Alberta, 2017d).

The oil sands are made up of sand, clay, water and bitumen (Government of Alberta, 2017b). Bitumen is a thick, heavy oil which requires refining and upgrading before being used as gas or diesel (Government of Alberta, 2017b). Due to degradation through organic processes, the thick bitumen is hydrogen deficient. Therefore, upgrading involves adding hydrogen or removing carbon to lighten the hydrocarbon. Upgrading also includes removing bitumen impurities (i.e., oxygen, metals, etc.) (Government of Alberta, 2017a), and requires large amounts of water which, after processing, contains various chemicals and contaminants. This contaminated water is pumped into tailings ponds where particulates settle out, and the water is reused for extraction. Eventually, these ponds are retired. According to legislation, these areas need to be reclaimed and returned to the government in a pre-mined equivalent land capability. Only once reclamation has occurred, will the companies receive a certificate stating that they met the criteria for land reclamation (Province of Alberta, 2016).

Reclamation requires a minimum of 1.0 m of soil or other suitable material to be used to cover the retired tailings pond, which then needs to be planted according to revegetation guidelines

(Government of Alberta, 2013). In the case of oil sands, many companies choose to cap the ponds using the overburden that was removed prior to mining. Sometimes geotextiles are used as a barrier between the pond's particulates and the overburden. However, reclamation does not address the leftover impurities found in the tailing sediment. Remediation goes one step further to remove and reduce contamination and concentrated impurities present in the tailings. In the past, a study was carried out where plants were seeded directly on the mine tailings to prevent erosion of the fine particulates; however, the tailings proved to be too harsh for the plant species (Lesko, 1974).

2.4.2 Metals

Remediating metal contaminated soil is different from remediating benzene or hydrocarbon contaminated soil because metals do not degrade. One way of remediating metal affected soil is by planting metallophytes, plants which can tolerate high levels of certain metals (Antonovics et al., 1971; Baker, 1987; Dahmani-Muller et al., 2000; Baker et al., 2010). Metallophytes have three strategies: exclusion, indication, and accumulation (Memon and Schröder, 2009; Ali et al., 2013). Excluders have mechanisms to avoid metal uptake, whereas indicators and accumulators absorb metals. A hyperaccumulating plant is a rare kind of accumulator. These plants can accumulate more than 1 % of their dry biomass in metals (Raskin et al., 1997; Memon and Schröder, 2009), or approximately 100-times what a regular plant would take up (Baker and Brooks, 1989). Conversely, McIntyer (2003) argues that different hyperaccumulating thresholds exist for different metals. For example, since cadmium (Cd) is not a macro or micronutrient and is toxic at low levels, a plant which can accumulate 0.01 % Cd in its biomass is considered a hyperaccumulator compared to other plant species. As noted by Sarma (2011), there are about 500 known hyperaccumulating plants; however, most hyperaccumulating plants only target one metal (Table 2.1). Very few multi-metal hyperaccumulators are known (Gerhardt et al., 2009). Examples of multi-metal hyperaccumulators are *Sedum alfredii* (Hance), which hyperaccumulates zinc (Zn) and Cd (Yang et al., 2004) and *Helianthus annuus* L. which hyperaccumulates Cd, chromium (Cr), and arsenic (As) (Cutright et al., 2010). Another way of increasing the amount of metals accumulated is by using a chelating agent, such as EDTA, on the soil making metals more bioavailable. However, this can increase the risk of groundwater contamination (Lombi et al., 2001; Wenzel et al., 2003).

Table 2.1. Examples of metal hyperaccumulating plants

Scientific Name	**Common name**	**Metal Accumulated (mg kg^{-1})**	**References**
Alyssum lesbiacum (Candargy) Rech.f.	Madwort	Ni: 23 000	(Küpper et al., 2001)
Amaranthus virdis L.	Slender amaranth	Cr: 2600	(Liu et al., 2008)
Arabidopsis halleri L.	Creeping rice paddy mustard	Zn: 16 500	(Bert et al., 2000)
Hesperis persica Boiss.	*	As: 1500	(Karimi et al., 2009)
Isatis cappadocica Desv.	*	As: 3000	(Karimi et al., 2009)
Nicotiana tabacum L.	Tobacco	Cd: 16 220	(Mench et al., 1989)
Pteris vittata L.	Brake fern	As: 22 600	(Ma et al., 2001)
Solanum nigrum L.	Black nightshade	Cd: 168	(Sun et al., 2006)
Stanleya pinnata (Pursh) Britton	Desert princesplume	Se: 12 700	(Galeas et al., 2007)
Streptanthus polygaloides A.Gray	Milkwort jewelflower	Ni: 14 800	(Reeves et al., 1981)
Thlaspi caerulescens J.Presl & C.Presl	Alpine penny-cress	Zn: 18 455 Cd: 28 050	(Brown et al., 1994; Lombi et al., 2000)
Thlaspi praecox Wulf.	Early penny-cress	Zn: 14 590 Cd: 5960 Pb: 3500	(Vogel-Mikuš et al., 2005)

* No common name was found for these plant species

Effective accumulating plants ideally possess the following characteristics: fast growing, deep roots, high biomass production, easy to harvest, and tolerate and accumulate multiple metals in the aerial biomass (Clemens et al., 2002). However, most hyperaccumulating species are slow growers and do not produce a lot of biomass (Yang et al., 2005). Thus far, there are no known plant species which exhibit all the desired characteristics. Some plants which produce substantial biomass, such as wetland species, can still be used for metal phytoremediation even if the accumulation factor is low. The low accumulation factor is compensated for with the high biomass (Deng et al., 2004). This increases the number of plant species that can be chosen for phytoremediation, making it easier to match a plant's abilities to the contaminated area. Occasionally, plants used in phytoremediation can be of economic benefit if they are capable of accumulating high amounts of metals in their aboveground biomass, as the biomass can then be "mined" for these accumulated metals (Robinson et al., 1997).

Plants that are not resistant to metal contaminated soils may be physically affected by the metals present. Lead (Pb), for example, can lead to phytotoxicity by obstructing or altering chloroplast structure, chlorophyll synthesis, and electron transport (Kumar et al., 2012). Chlorophyll content can therefore be used as a plant health indicator since low chlorophyll levels indicate an unhealthy plant (Burton et al., 1986). Some plants have developed coping mechanisms such as vacuole storage, cell wall binding sites, and restricting metal ion transport (Ernst et al., 1992; Hall, 2002; Windham et al., 2003; Memon and Schröder, 2009) as a form of mitigating metal contaminated soil conditions.

Plant metal tolerance can be interpreted a couple of ways. Tolerance can refer to a species in an area that is not susceptible to the toxicity symptoms that those around it exhibit, or it can refer to a subset of individuals in a species or population that are able to withstand higher metal toxicity than its relatives (Antonovics et al., 1971). A few studies found that plant resistance to metals increased in the presence of salts. For example, *Spartina maritima*'s (Curtis) Fernald resistance to nickel (Ni) and cobalt (Co) increased in the presence of sodium chloride (NaCl) at 0.2 M (Mesa et al., 2015), and *Tamarix smyrnensis* Bunge plants better resisted Cd when watered with a 3 % NaCl solution compared to those watered with 0 and 0.5 % NaCl solution (Manousaki et al., 2008). Many contaminated sites have multiple contaminants involving metals and salts like what can be found in oil sand tailings. If combinations of contaminants can promote plant growth, then co-

contamination could be exploited, decreasing the amount of time required to remediate an area. More studies are required to verify if this is feasible.

2.4.3 Salts

Salt phytoremediation is different from metals since the plant does not necessarily accumulate salt, but increases the rate at which calcite is dissolved. However, halophytes, plant species that are very salt tolerant, can sometimes accumulate sodium (Na) and other salt ions (Qadir et al., 2005). Blum (1988) noted three main obstacles that plants face in saline soils: drought, ion toxicity, and poor mineral nutrition. Plants growing in highly saline soils exhibit nutrient imbalances, as some nutrients' uptake will increase while others decrease (Alam, 1999). This imbalance exacerbates the fact that saline soils tend to have poor fertility (Qadir and Schubert, 2002). Productivity of saline and sodic soils can be improved through excessive water flushing, which is not always feasible, or by displacing Na ions by increasing the amount of soluble calcium (Ca) available (Qadir et al., 2005). One drawback to flushing soil is that salt ions can travel through the soil and contaminate groundwater. Other options for remediating saline soils include physically removing the salt crusts from the soil surface (Qadir et al., 2000), or through the use of porous ceramics, whereby salts accumulate in the pores removing ions from the soil. The ceramics are reusable and optimal salt accumulation is maintained through rinsing (Jalila et al., 2016).

2.5 Remediation using native plants

When creating a phytoremediation plan, it is important to consider using native plants whenever possible to minimize the potential of introduced species, and to minimize disturbances and changes to the local flora and fauna (Timmis and Pieper, 1999). A non-native species can become invasive (Mani and Kumar, 2014) lowering biodiversity, highlighting the practicality and importance of considering native species (Memon and Schröder, 2009), especially pioneering species. Additionally, native species are usually more genetically diverse and adapted to the climatic conditions of the area compared to widely available agricultural crops that are normally chosen (Brown and Johnston, 1976). Arctic and sub-arctic plant species are cold hardy, can tolerate low nutrient conditions, and will not become weeds (Johnson et al., 1976). These characteristics are imperative when considering reclamation and remediation in the sub-arctic climate of northern Alberta, where the oil sands are found.

There have been numerous reports stating that native plant species surrounding a contaminated site began recolonizing the disturbed area before any remediation or reclamation

action was taken (Skousen et al., 1990, 1994; Blain et al., 2017). For example, one of the first oil sand extraction sites, Bitumount Provincial Historic site located in northern Alberta, Canada, was abandoned in the late 1950's and left untouched. However, plants native to the area slowly moved back in and have recolonized the disturbed site despite elevated hydrocarbon concentrations (Blain et al., 2017; Government of Alberta, 2017e). No reclamation or remediation efforts at the site have been made.

2.6 Scope of Work

The oil sand tailings ponds in Fort McMurray, Alberta contain Cd, Cu, Cr, Ni, salts and other contaminants. According to the list of the most toxic compounds and elements of the Agency for Toxic Substances and Disease Registry (2017), Cd ranks seventh, Ni 57th, Cr 78th, and Cu 118th. Metals, in general, pose health problems to humans and other animals. Cadmium exposure has been linked to lung, prostate and renal cancers in animals as well as humans (Waalkes, 2003), and the aforementioned metals can be found at elevated levels in the leftover mine tailings. Similarly, salt compounds which include chloride and sulfate can be found in high concentrations throughout the oil sand mine tailings (MacKinnon et al., 2001; Renault et al., 2004).

Many plant species have been used for reclamation in Canada; however, little information exists on how well these plants perform under metal and salt stress. To increase phytoremediation efficiency of metals and salts, native plants require screening for resistance to these compounds. Through screening, improved guidelines can be developed to better match plant species and contaminants, decreasing the time required to phytoremediate an area.

The current project assessed reclamation approved Canadian native plant species for their ability to germinate and grow when exposed metal and salt compounds present in oil sand mine tailings.

3.0 ASSESSING METAL AND SALT RESISTANCE OF CANADIAN NATIVE PLANT SPECIES USING GERMINATION, AND ROOT AND SHOOT LENGTHS AS STRESS INDICATORS

3.1 Preface

The oil sand tailings ponds in Fort McMurray, Alberta, Canada contain metal, salt, hydrocarbon and naphthenic acid concentrations that exceed environmental safety guidelines. These large land areas need to be reclaimed and returned to the government in an equivalent land capability. Generally, once the ponds have been cleared of water, overburden removed prior to mining and peat are used to cap the tailings. However, this does not address the leftover impurities found in the tailing sediment that may move downslope through the soil or be taken up by plants whose roots have penetrated through the overburden-peat cap. Phytoremediation is a technology that uses plants and their associated microorganisms to uptake, breakdown and immobilize target soil contaminants. It is important to first consider plant species native to the region to minimize the introduction of potentially invasive species. Native plant species are acclimatized to the area and soil conditions and will not become weeds. Numerous plant species native to western Canada are used in reclamation; however, little information is available on their resistance to the metals and salts found in the oil sand mine tailings. Therefore, the objective of this study was to survey reclamation approved plant species native to western Canada for their ability to germinate and grow under metal and salt stress.

3.2 Abstract

Phytoremediation is a green technology where plants and their associated microorganisms uptake, breakdown or immobilize target soil contaminants. The objective of this study was to assess Canadian native plant species used in reclamation for their ability to germinate and grow under metal and salt contaminated conditions, evaluating their phytoremediation potential. Five plant species native to western Canada: *Achillea millefolium* (common yarrow), *Astragalus canadensis* (Canadian milkvetch), *Calamovilfa longifolia* (Prairie sandreed), *Koeleria macrantha* (Prairie Junegrass), and *Vicia americana* (American vetch) were screened for resistance to metals and salts found in oil sand mine tailings. Semi-solid water agar (0.75 % w/v) was contaminated with either a metal [$Cd(NO_3)_2$, $Cr(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$] or salt (KCl, NaCl, K_2SO_4, Na_2SO_4) and then planted with surface sterilized seeds. Seed germination was unaffected by the metal concentrations (0, 10, 20, 50 mg kg^{-1}); however, plant species exhibited significant decreases in root and shoot growth as metal concentrations increased. Unlike metals, salts tended to inhibit germination and root and shoot growth as concentrations increased (0, 1000, 10 000, 100 000 mg kg^{-1}); however, the lowest concentration of salt generally did not significantly affect these parameters. Of the five plant species assessed, *A. millefolium* had the best overall performance with germination, and root and shoot lengths. Collectively, the results indicate the tested plant species were capable of germinating in metal and salt contaminated medium, recognizing that salt levels were above what is normally observed in soil.

3.3 Introduction

Phytoremediation is a green technology gaining in popularity. It is the use of plants and their associated microbes to remediate contaminated soils (Salt et al., 1995; Zhang et al., 2013) and costs a fraction of other remediation options (Grommen and Verstraete, 2002). In the Athabasca oil sands, tailings ponds are reservoirs for water used in the bitumen extraction process. Sand, silt, clay, and impurities released during bitumen extraction are also found in these ponds. Reclamation of the ponds occurs once they are decommissioned and drained (Province of Alberta, 2016). However, chemicals and other contaminants remain under the overburden, which is the material most often used to cap decommissioned ponds (BGC Engineering Inc., 2010).

Oil sand tailings ponds are ideal locations for phytoremediation since it is more economical than chemical or physical cleaning of the soil, especially given the large-scale of the project. Plant roots can help stabilize pond tailings which reduces erosion (Johnson et al., 1993), and the added organic matter from plant biomass improves nutrient quality and soil structure, promoting overall plant growth (Gosselin et al., 2010). However, phytoremediation is a longer process than chemical or physical remediation and requires upkeep (Cunningham and Ow, 1996).

Considering native plants is important when planning a phytoremediation project to minimize potential invasive species (Timmis and Pieper, 1999; Mani and Kumar, 2014). While a plant species may be an efficient phytoremediator, it can be detrimental to the surrounding environment if they outcompete native flora. Ideally a reclaimed or remediated area reflects the pre-disturbance flora. Germination studies are required when testing new plant species or assessing previously untested contaminants to determine if the species are appropriate for growing in contaminated soil (Kranner and Colville, 2011). Seed dormancy can be a large obstacle to overcome since it is an adaptive strategy for germinating under the most optimal conditions (Nikolaeva, 1977; Bewley and Black, 1982).

This study was designed to determine the best assay method for seed germination, and determine potential remediation candidates from a pool of available plant species based on their germination and growth when exposed to various concentrations of metals and salts. The specific objectives of this study were to: 1) compare three germination assays; 2) quantify germination inhibition concentrations for metals and salts present in oil sand tailings; and, 3) quantify inhibitory or stunting effects on the radicle and plumule from metals and salts.

3.4 Materials and Methods

3.4.1 Plant selection

Plant species selection was based on government documents and industry reports focused on reclamation and revegetation of the oil sands and soils of Alberta (Government of Alberta, 2001; Gosselin et al., 2010; Smreciu et al., 2013) and on seed availability. A master list was created using priority lists of species found in the native plant revegetation guidelines of Alberta (Government of Alberta, 2001); the forbs, grasses and shrub lists in boreal plant species for reclamation of Athabasca oil sands disturbances (Smreciu et al., 2013); and the list of plant species used in the reclamation of the oil sands of Alberta (Gosselin et al., 2010). When selecting species for this experiment, tree species were excluded due to time constraints. Many species suggested for reclamation were not commercially available therefore limiting testable species. Alberta Innovates, a project partner, provided seed samples of some species that were unavailable from other distributors. However, due to the small number of seeds provided, only a preliminary germination test was conducted for these species.

Little to no literature was found for many Canadian approved reclamation plant species; however, *Eleocharis acicularis* (L.) Roem. & Schult., needle spikerush, appeared in the literature frequently. *Eleocharis acicularis* was able to hyperaccumulate copper (Cu), zinc (Zn), arsenic (As), and cadmium (Cd) in a stream environment (Sakakibara et al., 2011), and iron (Fe), lead (Pb), Zn, manganese (Mn), chromium (Cr), Cu, and nickel (Ni) at an abandoned mine (Ha et al., 2009).

3.4.2 Plant background of select species

Achillea millefolium L., common yarrow, a member of the Asteraceae family, is considered ubiquitous in its habitat (Smreciu et al., 2013) and is found throughout North America and in parts of Europe (Khela, 2012). *Achillea millefolium* has colonized reestablished plots at Syncrude and Suncor, which are oil sand extraction and processing sites located in Fort McMurray, Alberta, Canada (Geographic Dynamics Corp., 2006). These sites have tailings ponds which, among other things, contain various metals. Soudek et al. (2010) found that *A. millefolium* was able to accumulate varying levels of Cr, Pb, and Zn however the accumulation was not high enough for the plant to be considered a hyperaccumulator. Newly reclaimed tailings dykes at Suncor in the late 1970's also found *A. millefolium* invading and colonizing the site (Hardy BBT Limited, 1990), accounting for approximately 17 % of plant cover. *Achillea millefolium* has been used in the past as an air pollution indicator (Pilegaard, 1976), especially for metals (Radulescu et al., 2013).

Pilegaard and Johnsen (1984) found that Cu and Pb air content could be adequately monitored using *A. millefolium*. *Achillea millefolium* also readily took up Cd without any visual defects (Chizzola, 2005); however, the highest test treatment in Chizzola's (2005) study was 10 mg kg^{-1} which, while above the recommended environmental value, may not adequately determine *A. millefolium*'s resistance level. According to Hanslin and Eggen (2005), *A. millefolium* is moderately salt tolerant which refers to a seed germination between 20 and 60 % in the presence of 200 mM of sodium chloride (NaCl).

Astragalus canadensis L., also known as Canadian milkvetch, is a member of the Fabaceae family. As a member of the Fabaceae family, it has the capacity to form a symbiotic relationship with bacteria (i.e., *Rhizobium* spp.). These bacteria are known for their nitrogen fixing ability and this relationship may help *A. canadensis* overcome environmental stressors such salt and metal contamination found in the soil (Suárez et al., 2008; Wani and Khan, 2013). *Rhizobium* spp. have also been associated with Ni soil remediation. When used as a bio-inoculant, *Rhizobium* sp. increased nodulation, seed protein and yield, and decreased Ni uptake in lentils compared to plants grown without the inoculant (Wani and Khan, 2013).

Calamovilfa longifolia (Hook.) Scribn., Prairie sandreed, is a member of the Poaceae family. Due to *C. longifolia*'s large rooting system it is an ideal candidate for erosion control at sandy sites (USDA NRCS, n.d.). *Calamovilfa longifolia* is known for its drought tolerance (USDA NRCS, n.d.), however no soil moisture levels were associated with this claim. When exposed to decreased water potential (i.e., salt solution), *C. longifolia* germinated at lower potentials than other species (Mollard and Naeth, 2015) indicating potential resistance to salts.

Koeleria macrantha (Ledeb.) Schult., Prairie Junegrass, like *C. longifolia* is also a member of the Poaceae family. Wang et al. (2011) found that some varieties of *K. macrantha* better tolerated NaCl and suggested that this species be bred selectively for higher salinity tolerance. *Koeleria macrantha* is also known for its tolerance of extreme environmental temperatures, varying altitudes, and its ability to thrive under water stress (USDA NRCS, n.d.).

Vicia americana Muhl. ex Willd, American vetch, is a member of the Fabaceae family. Like *A. canadensis*, *V. americana* can form a symbiosis with *Rhizobium* spp. which may provide a competitive advantage in the presence of environmental stress, in addition to improving soil quality with its nitrogen fixation capacity. *Vicia americana* has been documented colonizing disturbed areas (Pahl and Smreciu, 1999) making it an good candidate to study for remediation.

3.4.3 Seed germination trials

Before testing germination under stress conditions, a baseline was required to determine seed response under neutral conditions. Seed germination was assessed using a modified Warman (1999) germination test. Briefly, 10 seeds of one species were placed in a sterile Whatman 41 filter paper lined 8 cm petri dish with 5 mL of deionized water. There were three concurrent replicates for each species (total 30 seeds). Plates were covered, placed in the dark, and watered every 2-3 d to keep paper moist. Seeds were incubated for 14 d at 23 °C. The number of germinated seeds was recorded every day for the first 10 d, and then twice thereafter (Table 3.1). Subsequent germination trials were carried out to confirm the consistency of germination. *Astragalus canadensis*' initial germination was low, but was consistently high in subsequent trials. *Elymus canadensis* and *Festuca campestris*' germination was inconsistent through multiple trials.

Table 3.1. Total germination of selected Canadian plant species after 14 d incubation at 23°C

Scientific Name	Common Name	Seed Source	Total Germination (%)
Achillea millefolium L.	Common yarrow	Telfer Seeds	80
Astragalus canadensis L.	Canada milkvetch	Telfer Seeds	27
Bromus ciliatus L.	Fringed brome	Telfer Seeds	37
Calamovilfa longifolia (Hook.) Scribn.	Prairie sandreed	Telfer Seeds	83
Chamerion angustifolium L. Holub	Fireweed	Alberta Innovates	7
Deschampsia caespitosa (L.) P. Beauv.	Tufted hair grass	Telfer Seeds	3
Distichlis stricta (Torr.) Rydb.	Inland saltgrass	Telfer Seeds	0
Elymus canadensis L.	Canada wild rye	Telfer Seeds	70
Elymus innovatus (Beal) Pilg.	Hairy wildrye	Telfer Seeds	3
Festuca campestris Rydb.	Rough fescue	Telfer Seeds	53
Gaillardia aristata Pursh	Blanket flower	Alberta Innovates	33
Hedysarum alpinum L.	Alpine sweet vetch	Alberta Innovates	63
Hedysarum boreale Nutt.	Boreal sweet vetch	Alberta Innovates	73
Koeleria macrantha (Ledeb.) Schult.	Prairie Junegrass	Telfer Seeds	57
Lathyrus ochroleucus Hook.	Pale vetchling	Alberta Innovates	27
Lathyrus venosus Muhl. ex Willd	Veiny pea	Alberta Innovates	0
Spartina pectinate Bosc ex Link	Prairie cordgrass	Telfer Seeds	7
Sporobolus cryptandrus (Torr.) A.Gray	Sand dropseed	Telfer Seeds	13
Vicia americana Muhl. ex Willd	American vetch	Telfer Seeds	73

Of the tested species, *C. longifolia*, had the highest germination with 83 % and was used to test the three assay methods: solid 1.5 % (w/v) water agar, semi-solid 0.75 % (w/v) water agar, and

wetted filter paper. Seeds were planted in groups of 10, with three concurrent replicates. Comparisons of surface disinfected and non-disinfected seeds was also carried out using a complete block design with three replicates per treatment (Appendix A). The disinfection procedure is described below. The assay method with the highest germination was selected for the subsequent study.

Seed surface disinfection was adapted from Abdellatif et al. (2009). Seed disinfection took place in a biosafety cabinet. In short, seeds were agitated in 65 % ethanol for two minutes and left to soak one minute in the ethanol. Seeds then soaked for five minutes in a 10 % sodium hypochlorite solution, and were agitated for the first 30 seconds. Seeds were rinsed 7-10 times in sterile deionized water. Seed disinfection was assessed by pipetting and spreading 100 µL of the final sterilized deionized water wash onto 1/10 tryptic soy agar (TSA) plate that contained cyclohexamide to inhibit fungal growth. The TSA plate with the final wash water was then incubated at 28.0 °C for 72 hours. Any bacterial growth on these plates was noted. Fungal growth was not observed.

Surface disinfected *C. longifolia* seeds were planted on solid water agar, 1.5 % (w/v) (Walley and Germida, 1997), semi-solid water agar, 0.75 % (w/v) (ElAafi et al., 2012), and wetted filter paper (Warman, 1999). The petri plates were placed in the dark at 23 °C for 14 d at which time the number of germinated seeds was counted.

3.4.4 Seed priming for low germinators

Many of the tested seed species exhibited low total germination (<50 %) in the absence of stress possibly because they required a break in dormancy. Poor germinating species included: *B. ciliatus*, *D. caespitosa*, *E. innovatus*, *F. campestris*, and *S. cryptandrus*. Seeds of these species were osmoprimed and hydroprimed to try to increase germination. Polyethylene glycol (PEG) 8000 (-1.5 MPa) was used for osmopriming (Pill and Korengel, 1997). The solution was made using 354 g of PEG 8000 per 1 L of deionized water. Approximately 30 mL of seed was placed in a 50 mL conical Corning ™ Falcon ™ centrifuge tube and filled to the 50 mL mark with the PEG 8000 solution. Seeds were incubated at room temperature in the dark for 4 d. Seeds were then rinsed multiple times using deionized water to remove any residual PEG. Seeds were placed on sterile filter paper lined petri dishes, 10 per plate, in triplicate. Plates were watered as needed to keep paper moist. Hydropriming was carried out much the same way, however in place of

PEG 8000 deionized water was used. Hydroprimed seeds were also soaked for 4 d in order to best compare the two chosen methods of seed priming.

3.4.5 Germination under metal and salt stress conditions

Six different compounds: Cd, chloride, Cr, Cu, Ni, and sulfate, were assessed for seed germination inhibition. Each treatment had three replicates of 10 seeds each. Salt (SO_4^{-2}, Cl^-) and metal (Cd^{+2}, Co^{+2}, Cu^{+2}, Ni^{+2}) trials employed various concentrations. Metal and salt concentrations found in the oil sand tailings pond water exceed many Canadian and American environmental protection guidelines where chloride ranges from 80-540 mg L^{-1}, and sulfate ranges from 218-290 mg L^{-1} (Allen, 2008b). Sediment impurity concentrations can be estimated from the concentrations found in the tailings water (Tessier and Campbell, 1987; van Beelen et al., 2003). The salt concentrations used in the study treatments were higher than what was reported in the pond water; however, previous pilot studies showed that the lower concentrations did not inhibit germination or seed growth. Concentrations for chloride contaminated agar were 1000, 10 000, and 100 000 mg L^{-1} of agar, whereas sulfate concentrations were 1000 and 10 000 mg L^{-1}. All metals were added at the same concentrations: 10, 20, and 50 mg metal L^{-1} of agar, based on concentrations observed in the literature for similar plant types. Compounds were tested independently.

A modified seed germination test was used (Di Salvatore et al., 2008). Briefly, metal and salt contaminated semi-solid agar plates were made using a 0.75 % semi-solid water agar created with deionized or double deionized water and Difco ™ agar flakes (Sparks, MD, USA). Metal contaminated agar consisted of agar and 490 mL of double deionized water mixed in an acid washed media flask and autoclaved. The 10 mL of metal contaminants [cadmium nitrate [$Cd(NO_3)_2$], cobalt nitrate [$Co(NO_3)_2$], copper (II) nitrate [$Cu(NO_3)_2$], or nickel (II) nitrate [$Ni(NO_3)_2$] was filter sterilized using a 0.45 µm cellulose syringe filter with an acetate membrane (VWR International), added to the water agar and subsequently mixed. Salt contaminated agar was created using salt [sodium sulfate (Na_2SO_4), potassium sulfate (K_2SO_4), sodium chloride (NaCl), potassium chloride (KCl)] that was added to a graduated cylinder. Deionized water was added to reach the 500 mL mark and were then added to the agar. Agar and the salt solution were added to the media flask which was subsequently autoclaved. Petri plates were poured in a biosafety cabinet. Concentrations of chemicals used was based on pilot experiments and literature (Nedelkoska and Doran, 2001; Ghosh and Singh, 2005; Bai et al., 2014; Li et al., 2016).

Seeds were surface disinfected based on Abdellatif et al.'s (2009) protocol. In brief, seeds were agitated in 95 % ethanol for 10 seconds, rinsed with sterilized deionized water, then left to soak for three minutes in a 10 % sodium hypochlorite solution. Seeds were rinsed three to five times in sterile deionized water. Seed disinfection verification and planting were performed as previously described.

Disinfected seeds were placed in petri plates and incubated at 23 °C for 7 d. Germinated seeds (i.e., a radicle or plumule that was greater than 1 mm) were then counted and root and shoot length was recorded. Root and shoot length was measured using WinRHIZO ™ 2013 (Regent Instruments Inc., Québec City, QC, Canada), a root imaging analysis software (Fig. 3.1). Only roots and shoots greater than 3 mm were analyzed due to difficulties encountered when scanning small seedlings (i.e., seeds were too large to lay flat on the scanning tray or would spin and float during scanning).

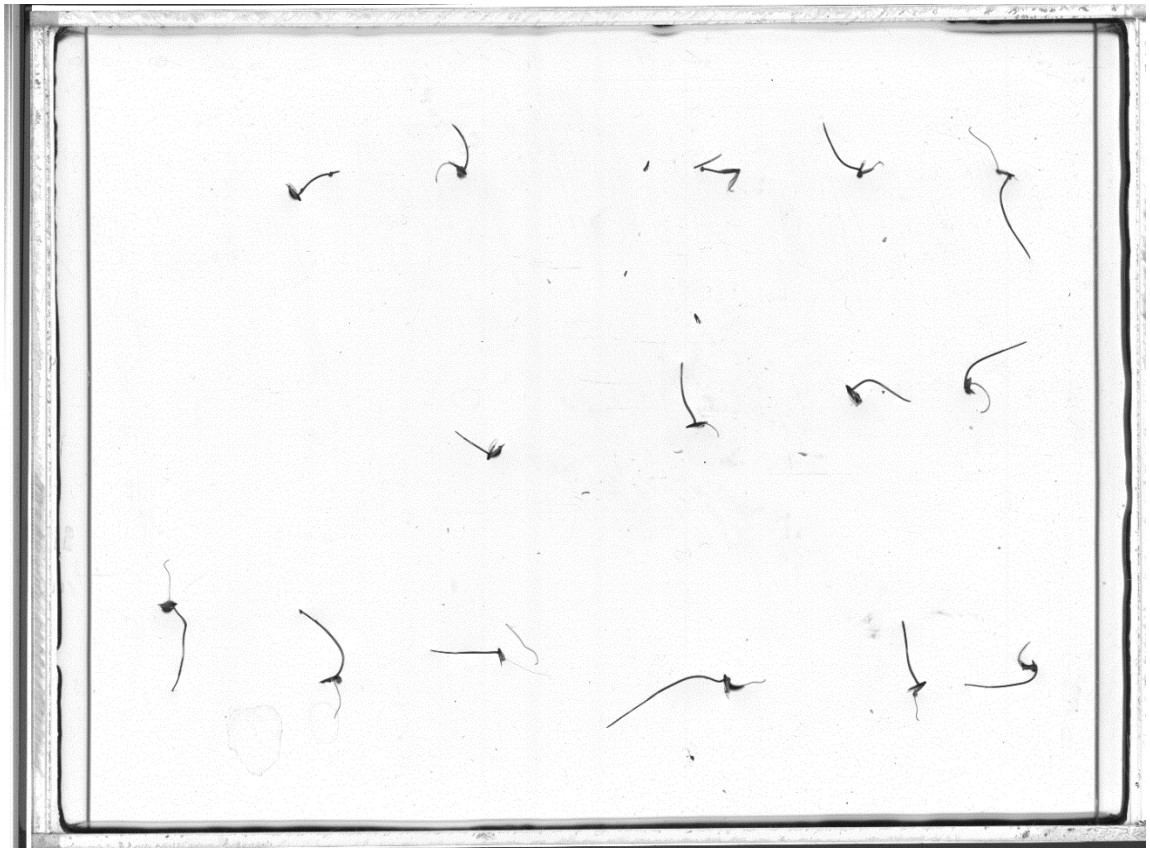

Figure 3.1. WinRHIZO ™ scan of *Koeleria macrantha* exposed to 10 mg kg^{-1} $Ni(NO_3)_2$; each horizontal grouping of seeds represents plate replicates (Top row: Plate A, middle row: Plate B, bottom row: Plate C).

3.4.6 Statistical analysis

Statistical analysis was performed using SAS ® 9.4 (SAS Institute Inc., 2016). A one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test were used to determine significant changes in germination, and growth of roots and shoots between treatments. Sattherwaite's degrees of freedom was used. Data included zero values for seeds that did not germinate to ensure equal weight between replicates. Normality of data was verified using the Shapiro-Wilk test.

The germinated seed replicates of *A. millefolium* exposed to 20 mg kg^{-1} Ni got mixed together in error; therefore, the scanned seeds were assigned a replicate based on random number generation. The following results for *A. millefolium* exposed to 20 mg kg^{-1} Ni are for trend purposes only as statistical analysis was not possible.

3.5 Results

Calamovilfa longifolia was used as the growth media test plant since it consistently had the highest total germination. The total germination for semi-solid agar and wetted filter paper was very similar with 80 % and 83 %, respectively, whereas solid water agar supported a slightly lower germination at 70 %. Since wetted filter paper and semi-solid water agar produced similar germination results, medium selection was done based on ease of use. Semi-solid water agar was easier to prepare than wetted filter paper. The osmopriming and hydropriming of poorly germinating plant species did not increase germination; therefore, those species were not used in the subsequent germination and growth tests.

3.5.1 Germination

Germination of *A. canadensis*, *A. millefolium*, and *C. longifolia* was uninhibited by the metal treatments. *Koeleria macrantha* had a significant (p<0.05) decrease in germination when exposed to Cd and Cu at both 20 and 50 mg kg^{-1}, but remained unaffected at 10 mg kg^{-1}. *Vicia americana*'s germination in 50 mg kg^{-1} Cr was significantly (p<0.05) inhibited as compared to the control (Table 3.2).

None of the plant species' germination was affected when exposed to 1000 mg kg^{-1} NaCl or either treatment of 1000 mg kg^{-1} sulfate. However, *V. americana* did exhibit significant (p<0.05) germination inhibition when exposed to 1000 mg kg^{-1} KCl. All species showed significant (p<0.05) germination inhibition when exposed to 10 000 and 100 000 mg kg^{-1} chloride. *Astragalus canadensis*' germination remained the same when exposed to 10 000 mg kg^{-1} sulfate; however, *C.*

longifolia, *K. macrantha*, and *V. americana*'s germination was significantly (p<0.05) inhibited. Germination of *A. millefolium* was significantly inhibited when exposed to 10 000 mg kg^{-1} K_2SO_4, but not that of Na_2SO_4 (Table 3.2).

3.5.2 Root and shoot lengths

Root length was significantly (p<0.05) shorter for all species when exposed to Cd (10, 20, 50 mg kg^{-1}) except for *V. americana* whose root lengths were uninhibited when exposed to 10 mg kg^{-1} Cd (Table 3.3). In contrast, *A. canadensis* and *A. millefolium* were the only species to show significant (p<0.05) shoot inhibition in 10 mg kg^{-1} Cd. *Vicia americana* shoots remained uninhibited when exposed to 20 mg kg^{-1} Cd (Table 3.4).

Astragalus canadensis, *A. millefolium*, and *C. longifolia* all had stunted roots (p<0.05) when exposed to 10, 20, and 50 mg kg^{-1} Ni. *Koeleria macrantha*'s roots were significantly inhibited by 50 mg kg^{-1} Ni; however, *V. americana* roots remained uninhibited. *Achillea millefolium* shoots were significantly (p<0.05) shorter than the control's shoots when exposed to 10, 20, and 50 mg kg^{-1} Ni. *Koeleria macrantha* roots showed Ni sensitivity when exposed to 50 mg kg^{-1} Ni.

Achillea millefolium and *C. longifolia* were the only species to exhibit significant (p<0.05) root inhibition when exposed to Cr. Where *A. millefolium* expressed shorter roots in 20 and 50 mg kg^{-1} Cr, *C. longifolia* only exhibited root inhibition in 10 mg kg^{-1} Cr. *Achillea millefolium* shoots significantly (p<0.05) decreased in the presence of 50 mg kg^{-1} Cr.

Roots exposed to Cu were significantly (p<0.05) shorter than the control for all species except for *A. canadensis* exposed to 10 and 20 mg kg^{-1} Cu. All Cu treatments significantly (p<0.05) inhibited shoots of *A. canadensis*, *A. millefolium*, and *K. macrantha*, whereas *C. longifolia* shoots were only significantly (p<0.05) inhibited by 50 mg kg^{-1} Cu.

Vicia americana expressed significant (p<0.05) root and shoot inhibition when exposed to 1000 mg kg^{-1} KCl, and all species exhibited significant (p<0.05) root and shoot stunting when exposed to salt concentrations exceeding 1000 mg kg^{-1}. *Astragalus canadensis* showed significant (p<0.05) shoot sensitivity when exposed to 1000 mg kg^{-1} NaCl. Similarly, *A. millefolium* shoots were significantly (p<0.05) stunted in 1000 mg kg^{-1} Na_2SO_4.

Table 3.2. Total germination of select Canadian native plant species grown in metal and salt contaminated semi-solid agar after 7 d in the dark at 23 °C

Treatment	Dose (mg kg^{-1})	Germination ± SD (%)*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control	0	47.5 ± 18.7 a	94.2 ± 10.0 a	73.3 ± 11.6 ab	61.8 ± 22.3 ab	66.4 ± 11.2 ab
KCl	1000	50.0 ± 10.0 ab	93.3 ± 11.6 ab	53.3 ± 15.3 abcde	43.3 ± 28.9 bcdef	20.0 ± 10.0 def
	10 000	0.0 ± 0.0 b	0.0 ± 0.0 c	3.3 ± 5.8 fg	0.0 ± 0.0 f	0.0 ± 0.0 f
	100 000	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 g	0.0 ± 0.0 f	0.0 ± 0.0 f
NaCl	1000	53.3 ± 20.8 a	93.3 ± 11.6 ab	70.0 ± 10.0 abcd	36.7 ± 5.8 bcdef	43.3 ± 5.8 bcde
	10 000	0.0 ± 0.0 b	0.0 ± 0.0 c	26.7 ± 12.5 efg	0.0 ± 0.0 f	0.0 ± 0.0 f
	100 000	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 g	0.0 ± 0.0 f	0.0 ± 0.0 f
K_2SO_4	1000	46.7 ± 20.8 ab	90.0 ± 10.0 ab	53.3 ± 5.8 abcde	60.0 ± 20.0 abc	53.3 ± 15.3 abcd
	10 000	13.3 ± 5.8 ab	66.7 ± 15.3 b	40.0 ± 17.3 cdef	13.3 ± 15.3 def	0.0 ± 0.0 f
Na_2SO_4	1000	50.0 ± 30.0 ab	83.3 ± 11.6 ab	43.3 ± 11.6 bcde	40.0 ± 17.3 bcdef	66.7 ± 5.8 abc
	10 000	13.3 ± 5.8 ab	83.3 ± 20.8 ab	33.3 ± 5.8 defg	0.0 ± 0.0 f	16.7 ± 5.8 ef
Cd	10	43.3 ± 15.3 ab	96.7 ± 5.8 a	70.0 ± 17.3 abcd	53.3 ± 11.6 abcd	66.7 ± 15.3 abc
	20	46.7 ± 15.3 ab	93.3 ± 5.8 ab	50.0 ± 20.0 abcde	20.0 ± 10.0 cdef	63.3 ± 15.3 abc
	50	60.0 ± 17.3 a	90.0 ± 10.0 ab	50.0 ± 10.0 abcde	0.0 ± 0.0 f	53.3 ± 5.8 abcd
Cr	10	36.7 ± 25.2 ab	96.7 ± 5.8 a	50.0 ± 0.0 abcde	93.3 ± 5.8 a	73.3 ± 15.3 ab
	20	43.3 ± 11.6 ab	100 ± 0.0 a	56.7 ± 25.2 abcde	93.3 ± 5.8 a	56.7 ± 15.3 abc
	50	50.0 ± 20.0 ab	100 ± 0.0 a	60.0 ± 17.3 abcde	90.0 ± 10.0 a	36.7 ± 15.3 cde
Cu	10	53.3 ± 5.8 a	96.7 ± 5.8 a	60.0 ± 0.0 abcde	43.3 ± 20.8 bcdef	83.3 ± 11.6 a
	20	36.7 ± 15.3 ab	100 ± 0.0 a	83.3 ± 5.8 a	6.7 ± 5.8 ef	66.7 ± 15.3 abc
	50	43.3 ± 25.2 ab	93.3 ± 5.8 ab	73.3 ± 11.6 abc	0.0 ± 0.0 f	70.0 ± 0.0 abc
Ni	10	36.7 ± 20.8 ab	96.7 ± 5.8 a	56.7 ± 20.8 abcde	50.0 ± 10.0 abcde	63.3 ± 5.8 abc
	20	36.7 ± 5.8 ab	100 ± 0.0 a	76.7 ± 5.8 abc	50.0 ± 17.3 abcde	60.0 ± 10.0 abc
	50	60.0 ± 10.0 a	93.3 ± 5.8 ab	73.3 ± 5.8 abc	50.0 ± 20.0 abcde	60.0 ± 10.0 abc

* Mean ± standard deviation (SD) where different letters in the same column are significantly ($p<0.05$) different using Tukey's HSD (n=3 for all treatments, except the Control where n=12)

Table 3.3: Root length of select Canadian native plant species grown in metal and salt contaminated semi-solid agar after 7 d in the dark at 23 °C measured using WinRHIZO™

Treatment	Dose (mg kg^{-1})	Root length ± SD (mm)*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control	0	5.1 ± 2.0 a	8.7 ± 1.4 ab	6.8 ± 2.1 a	6.1 ± 4.0abc	9.1 ± 5.8 a
KCl	1000	5.2 ± 1.3 ab	7.7 ± 1.9 ab	5.8 ± 2.1 ab	4.7 ± 3.3 abcd	1.7 ± 1.2 bc
	10 000	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	0.0 ± 0.0 c
	100 000	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	0.0 ± 0.0 c
NaCl	1000	1.7 ± 1.5 abcd	8.3 ± 1.0 ab	4.4 ± 1.7 abcd	1.4 ± 0.3 cd	2.8 ± 0.8 abc
	10 000	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	0.0 ± 0.0 c
	100 000	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	0.0 ± 0.0 c
K_2SO_4	1000	3.8 ± 1.1 abcd	7.1 ± 1.6 ab	4.8 ± 0.1 abc	5.5 ± 3.2 abcd	5.5 ± 3.2 abc
	10 000	0.3 ± 0.3cd	0.0 ± 0.0 c	0.3 ± 0.4 e	0.0 ± 0.0 d	0.0 ± 0.0 c
Na_2SO_4	1000	4.9 ± 1.8 ab	6.9 ± 0.8 ab	5.3 ± 1.3 abc	3.6 ± 1.1 bcd	3.6 ± 1.1 abc
	10 000	0.0 ± 0.0 d	0.0 ± 0.0 c	0.5 ± 0.8 de	0.0 ± 0.0 d	0.0 ± 0.0 c
Cd	10	0.0 ± 0.0 d	1.3 ± 1.0 c	1.4 ± 0.4 cde	0.0 ± 0.0 d	2.5 ± 0.9 abc
	20	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	1.1 ± 0.3 c
	50	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	0.3 ± 0.3 c
Cr	10	4.8 ± 2.8 abc	7.6 ± 0.8 ab	2.8 ± 1.5 bcde	10.8 ± 0.6 a	10.0 ± 2.8 ab
	20	5.1 ± 2.0 ab	6.5 ± 0.2 b	3.8 ± 0.5 abcde	9.4 ± 1.6 ab	7.9 ± 1.8 abc
	50	2.3 ± 0.7 abcd	0.5 ± 0.3 c	5.2 ± 2.3 abc	5.4 ± 0.8 abcd	2.8 ± 2.0 abc
Cu	10	5.8 ± 2.0 a	0.6 ± 0.6 c	0.0 ± 0.0 e	0.0 ± 0.0 d	1.2 ± 0.7 c
	20	2.1 ± 3.6 abcd	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	0.7 ± 0.8 c
	50	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d	0.4 ± 0.1 c
Ni	10	1.2 ± 0.7 bcd	2.0 ± 0.9 c	3.6 ± 1.7 bcde	2.9 ± 1.8 cd	7.0 ± 2.5 abc
	20	0.3 ± 0.2 cd	0.0 ± 0.0 c†	2.9 ± 0.9 bcde	1.2 ± 0.8 cd	4.4 ± 1.9 abc
	50	0.0 ± 0.0 d	0.0 ± 0.0 c	1.5 ± 0.9 cde	0.0 ± 0.0 d	2.7 ± 0.2 abc

* Mean ± standard deviation (SD) where different letters in the same column are significantly ($p<0.05$) different using Tukey's HSD (n=3 for all treatments, except the Control where n=12); † for trend purposes only since sample was mixed

Table 3.4: Shoot length of select Canadian native plant species grown in metal and salt contaminated semi-solid agar after 7 d in the dark at 23 °C measured using WinRHIZO™

Treatment	Dose (mg kg^{-1})	Shoot length ± SD (mm)*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control	0	8.2 ± 2.4 ab	24.5 ± 3.1 a	9.8 ± 2.6 a	10.8 ± 5.4 abc	7.9 ± 3.6 a
KCl	1000	11.5 ± 4.5 a	24.5 ± 4.5 abc	11.0 ± 3.1 a	7.9 ± 5.1 abcde	2.5 ± 0.8 bcd
	10 000	0.0 ± 0.0 d	0.0 ± 0.0 g	0.1 ± 0.2 e	0.0 ± 0.0 e	0.0 ± 0.0 d
	100 000	0.0 ± 0.0 d	0.0 ± 0.0 g	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 d
NaCl	1000	2.9 ± 2.5 cd	21.9 ± 2.6 abcd	8.8 ± 0.7 ab	4.6 ± 0.9 cde	3.8 ± 0.9 abcd
	10 000	0.0 ± 0.0 d	0.0 ± 0.0 g	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 d
	100 000	0.0 ± 0.0 d	0.0 ± 0.0 g	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 d
K_2SO_4	1000	8.8 ± 4.2 abc	22.7 ± 4.1 abc	7.5 ± 1.2 abcd	9.5 ± 4.3 abcd	9.5 ± 4.3 a
	10 000	0.0 ± 0.0 d	0.0 ± 0.0 g	3.2 ± 1.5 bcde	0.3 ± 0.5 e	0.3 ± 0.5 d
Na_2SO_4	1000	10.4 ± 4.2 ab	19.4 ± 1.7 cd	7.7 ± 3.3 abcd	6.5 ± 1.7 bcde	6.5 ± 1.7 abcd
	10 000	0.0 ± 0.0 d	0.0 ± 0.0 g	2.8 ± 0.4 cde	0.0 ± 0.0 e	0.0 ± 0.0 d
Cd	10	2.3 ± 1.1 cd	9.5 ± 1.4 e	8.5 ± 1.2 abc	3.7 ± 1.5 cde	8.2 ± 1.9 ab
	20	2.3 ± 0.6 cd	2.7 ± 0.5 fg	3.1 ± 1.4 bcde	0.6 ± 0.7 e	4.9 ± 1.9 abcd
	50	2.5 ± 0.5 cd	0.0 ± 0.0 g	2.2 ± 1.0 de	0.0 ± 0.0 e	1.1 ± 0.4 cd
Cr	10	6.3 ± 3.2 abcd	25.6 ± 0.4 ab	5.6 ± 0.9 abcde	16.6 ± 0.4 a	9.5 ± 1.2 a
	20	7.9 ± 2.6 abc	24.7 ± 1.4 abc	6.3 ± 2.7 abcd	16.4 ± 1.2 a	7.7 ± 1.9 abc
	50	5.9 ± 1.1 abcd	19.5 ± 1.3 bcd	8.2 ± 2.4 abc	15.2 ± 3.7 ab	4.8 ± 3.0 abcd
Cu	10	0.0 ± 0.0 d	6.0 ± 1.2 efg	5.6 ± 2.3 abcde	0.3 ± 0.3 e	7.6 ± 2.1 abc
	20	0.0 ± 0.0 d	2.2 ± 0.5 g	5.5 ± 1.8 abcde	0.0 ± 0.0 e	4.8 ± 2.5 abcd
	50	0.0 ± 0.0 d	0.1 ± 0.2 g	3.4 ± 1.8 bcde	0.0 ± 0.0 e	5.2 ± 2.0 abcd
Ni	10	5.8 ± 3.9 abcd	16.1 ± 2.3 d	6.4 ± 2.1 abcd	6.6 ± 2.5 bcde	8.0 ± 2.4 ab
	20	3.8 ± 0.2 bcd	8.4 ± 1.3 ef†	7.8 ± 0.8 abcd	4.4 ± 1.5 cde	7.1 ± 1.1 abc
	50	5.0 ± 1.2 abcd	4.7 ± 0.1 efg	7.0 ± 1.9 abcd	1.3 ± 1.2 de	5.8 ± 0.6 abcd

* Mean ± standard deviation (SD) where different letters in the same column are significantly ($p<0.05$) different using Tukey's HSD (n=3 for all treatments, except the Control where n=12); † for trend purposes only since sample was mixed

3.6 Discussion

Oil sand tailings pond sediment contains many compounds and elements which can be harmful to overall plant health and growth. The primary goal of this study was to evaluate plant species germination and growth in metals and salts found in oil sand mine tailings. More efficient remediation of metal and salt impacted soils can be achieved by increasing knowledge of germination resistance and growth of reclamation approved plant species. Many of the species were able to germinate in metal contaminated medium though, overall, root and shoot lengths were stunted. Seed germination and growth were unaffected by the lowest salt concentrations tested; however, seeds were sensitive to higher salt concentrations. *Calamovilfa longifolia* shoots and *V. americana* shoots and roots were less inhibited by metal treatments than was *A. canadensis*. *Calamovilfa longifolia* was the best performing species when looking at germination across treatments as it was the only species to germinate in chloride exceeding 1000 mg kg^{-1}. However, when considering total germination percentage and root and shoot lengths *A. millefolium* was the clear standout. Plant species had varying germination and growth responses, as expected, based on varying resistance levels. Further testing is required to determine if any of the species tested would be good candidates for remediating metal and/or salt contaminated soil.

Koeleria macrantha and three other grasses were exposed to NaCl. Germination was significantly lower in 5 mg kg^{-1} NaCl compared to the control, and almost no germination occurred at 20 mg kg^{-1} NaCl. Means were calculated by combining all plant species and varieties (Wang et al., 2011). My study's findings differ from Wang et al. (2011) where *K. macrantha* seeds exposed to KCl or NaCl at 1000 mg kg^{-1} had a no significant germination differences from the control. The difference in findings may be attributed to variation in seed lots, seed variety, genotype or phenotype. Phenotypic plasticity refers to the genetic make-up that can be influenced by environmental factors. Sometimes selection favors this plasticity singling out specific genotypes based on environmental conditions (Bradshaw, 1965; Schlichting, 1986). Given that there was no indication confirming that the seed in the experiment conducted by Wang et al. (2011) were identical to the ones used in my experiment, the differences observed may be attributed to the seeds' make-up. A small pilot was conducted prior to my study using lower salt concentrations, however, germination was unaffected by the concentrations tested by Wang et al. (2011).

In an assessment of grasses and forbs, *K. macrantha* had a high tolerance to chloride when exposed to 300 mg kg^{-1} NaCl; however it was more sensitive when grown in the same concentration

of magnesium chloride (Dudley et al., 2014). Tolerance levels were based on treatment percentages of the control germination and classed: 1-50 % is low, 51-80 % is medium, and 81-100 % is high. *Koeleria macrantha* had a medium salt tolerance when exposed to a 1350 mg kg^{-1} NaCl solution, and low tolerance to 3000 mg kg^{-1} NaCl (Dudley et al., 2014). Similar results were observed in my study where, using the same categorical scale, *K. macrantha* had 67 % germination than the control when grown in 1000 mg kg^{-1} NaCl, whereas in the same level of KCl 79 % of the control's germination was observed. Therefore, *K. macrantha* would be classified as moderately tolerant to chloride.

Germination of *C. longifolia* and *K. macrantha*, among others, was assessed under varying water potentials, and found results similar to those in my study (Mollard and Naeth, 2015). Germination decreased as negative water potential (salt content) increased, and *C. longifolia* was able to germinate at a lower water potential when other species could not. The delay in seed germination was something not explicitly examined in my study; however, *C. longifolia* did have a germination lag when exposed to salts but out-germinated many of the other species. *Calamovilfa longifolia* was the only species to germinate in the 10 000 mg kg^{-1} chloride contaminated medium at 3.3 % and 27 % in KCl and NaCl, respectively.

The treatment levels of Ni in my study were not found to significantly inhibit germination, though the percent germination tended to be lower than the control. Conversely, Ni ranging from 10-2000 µM (~0.6 to 117.4 mg kg^{-1}) significantly decreased the germination of halophyte *Salicornia ramosissima* J. Woods, however, *Atriplex halimus* L., also a halophyte, was unaffected (Márquez-García et al., 2013).

Plant roots were most sensitive to Cd and Cu, and least sensitive to Cr. The roots of lettuce, broccoli and tomato followed a similar trend (DiSalvatore et al., 2008). Comino et al. (2005) study found that Ni hyperaccumulator *Alyssum murale*'s Waldst. & Kit. shoots were significantly (p<0.05) inhibited at 100 mM NaCl (~5 g kg^{-1}); however, they found no interaction effect when *A. murale* was amended with both Ni and NaCl. Interestingly, Di Salvatore et al. (2008) evaluated plant roots to determine plant stress, where Comino et al. (2005) evaluated plant shoots. My study measured both roots and shoots since roots are important for nutrient and water uptake, whereas contaminants in shoots can be harvested and removed from the system.

3.7 Conclusion

Initially, I hypothesized that plants would not exhibit a change in germination when exposed to various metal and salt concentrations, and found that this was true for seeds exposed to metals. However, seeds exposed to salts had significantly lower germination rates as salt concentrations increased. Root and shoot lengths were inhibited by both metals and salts as concentrations increased, however roots were often more sensitive than shoots. Overall, the best plant performance was for *A. millefolium*. It had some of the highest germination rates for multiple contaminants, and had the longest roots and shoots of all species.

Further research is required to evaluate the plants tested in this study in metal and salt contaminated soil. Plants growing in contaminated soil may cause plants to germinate and grow differently than what they exhibited in the contaminated agar. Studying the plants' growth in a controlled environment is required to accurately determine whether these plant species are suitable for metal and salt soil remediation. Other methods used to break seed dormancy could also be explored in order to assess salt and metal resistance of the species that germinated poorly. Future work can also include assessing other plant species' germination and growth in the metals and salts evaluated in the agar study, and looking at plant resistance to metal mixtures, and metal and salt co-contamination. Small field trials may also be conducted to determine if the plant species are able to resist salt and metal stress while under varying environmental conditions.

4.0 ASSESSMENT OF CANADIAN NATIVE PLANT SPECIES RESISTANCE TO SALT AND METAL STRESS IN ARTIFICIALLY CONTAMINATED AGRICULTURAL SOIL: A GREENHOUSE STUDY

4.1 Preface

In the previous chapter it was shown that five tested plant species native to western Canada were capable of germinating and growing in metal or salt contaminated semi-solid agar. Results indicated plants were more resistant to the concentrations of metals tested than to salts based on the differences in germination rates. Further plant assessment was required to determine the phytoremediation potential of the plants in a more realistic setting; therefore, a greenhouse study was conducted. Seeds were planted in metal or salt contaminated soil and grown for 42 d post-emergence. Seed germination, and aboveground, belowground, and total biomass were measured and, based on these parameters, sensitivity of plants to contaminants was assessed.

4.2 Abstract

Native plants species are adapted to the local climate and soil conditions and have little chance of becoming a pest. Therefore, it is important to assess their abilities to assist in soil remediation. This study screened five plant species native to western Canada [*Achillea millefolium* (common yarrow), *Astragalus canadensis* (Canadian milkvetch), *Calamovilfa longifolia* (Prairie sandreed), *Koeleria macrantha* (Prairie Junegrass), *Vicia americana* (American vetch)] for their resistance to metals and salts found in oil sand mine tailings. In a growth chamber experiment, seeds were planted in metal [$Cd(NO_3)_2$, $Cr(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$] or salt (KCl, NaCl, K_2SO_4, Na_2SO_4) contaminated agricultural soil. Species were evaluated on their emergence and their biomass production. Plant biomass was measured 42 d post-emergence. *Astragalus canadensis*, *A. millefolium* and *C. longifolia*'s germination was not affected by the metal contaminated soil. Cadmium and copper (10, 20 mg kg^{-1}) significantly ($p<0.05$) inhibited *K. macrantha*'s germination and *V. americana*'s germination was significantly ($p<0.05$) inhibited by chromium (50 mg kg^{-1}); however, metal exposure had no effect on plant biomass for any of the tested species. Similarly, there was no significant differences in germination or biomass between plants exposed to 1000 mg kg^{-1} sulfate amended soil and the control. However, all plant species either failed to grow or were stunted in 1000 mg kg^{-1} chloride, and 5000 mg kg^{-1} sulfate and chloride amended soils. *Achillea millefolium* is being recommended for future studies since it had the highest biomass production and the best seed emergence of species evaluated.

4.3 Introduction

Soil contamination is an increasingly important global environmental issue mainly caused by the rapid expansion of industry and agriculture. The Canadian oil sands located in Fort McMurray, Alberta play an important role in the country's economy (Government of Canada, 2015); however, mining and processing the oil sands creates large amounts of contaminated water. Tailings ponds are where the water used to extract bitumen from oil sands is stored. Particulates settle out, and the water is reused. The extraction process concentrates impurities found in the bituminous sands. Some of these impurities include various metals (i.e., chromium, copper, vanadium) (Mahdavi et al., 2013), and salts (Renault et al., 1998).

Metals are not necessarily harmful, and many are essential micronutrients; however, micronutrients can become toxic at elevated levels. For example, chromium (Cr) has been known to inhibit photosynthesis impeding plant growth (Shanker et al., 2005), whereas excess iron (Fe) has been linked to increased oxidative stress (de Oliveira Jucoski et al., 2013). Metals can also move down the soil profile into groundwater, through mine waste water or mine tailings, which eventually reach larger water bodies, such as rivers (Chen et al., 2016; Roy et al., 2016). Remediating the soil to guideline approved metal levels is important for the safety of humans and the environment. Salts, on the other hand, are not toxic in the same way that metals are. Salts can negatively affect plants directly through ion toxicity (Greenway and Munns, 1980) or indirectly by decreasing the amount of water flowing through plant roots (Martínez-Ballesta et al., 2000).

Phytoremediation is one of the newest approaches to remove contaminants from soil, and stands out since it is less environmentally invasive and less expensive than other options (Grommen and Verstraete, 2002; Glick, 2010). Phytoremediation is a process where plants and their associated microbes uptake, breakdown, or immobilize target soil contaminants (Salt et al., 1995; Zhang et al., 2013).The success of a phytoremediation plan relies on the resistance of plants to the soil contaminants and, depending on the goal, the contaminant uptake ability. Determining resistance thresholds is an important step to ensure the growth and survival of the plants at the contaminated site. If a species is unsuccessful growing in controlled contaminated conditions, it is unlikely that the species will be successful under variable field conditions. In the field, contaminants are less bioavailable; however, there are unpredictable biotic and abiotic stressors, and large variabilities in contaminant distribution all of which contribute to failed phytoremediation field experiments (Gerhardt et al., 2009). Contaminant hotspots exist due to soil heterogeneity. Hotspots are areas

where the contaminant concentration is significantly higher than the surrounding soil (Ferro et al., 1999; ITRC, 2008), thus impeding plant growth. Hotspots are mitigated by physical removal and disposal of soil (USEPA, 2000), or through tillage of the hotspot with the surrounding soil, averaging the contamination over a larger area (Chaney et al., 2010). Regardless of hotspots, replanting and reseeding areas with poor plant growth is required for effective phytoremediation (Schnoor, 1997), and these costs need to be factored into the economic plan of the phytoremediation site (USEPA, 2000).

It is important to evaluate both aboveground and belowground growth for visible negative effects when screening plants for potential phytoremediation effectiveness. Plant roots are an essential part of phytoremediation. Roots receive the majority of a plant's nutrients and water, and are where phytoremediation usually occurs. For example, roots exude compounds which can break down contaminants or change them into bioavailable forms (Nepovím et al., 2004; Sun et al., 2010; Wang et al., 2012) and roots can uptake various contaminants, effectively removing them from the soil system (Borgegard and Rydin, 1989; Yifru and Nzengung, 2006; Whitfield Åslund et al., 2008).

The purpose of this experiment was to test Canadian native reclamation approved plant species for their ability to germinate and grow in salt or metal contaminated soil. The specific study objectives were to: 1) determine and compare seed emergence of reclamation approved plant species in a metal or salt contaminated soil medium; and 2) determine and compare differences in aboveground, belowground and total biomass of plants exposed to various concentrations of metals and salts in soil.

4.4 Materials and Methods

4.4.1 Collection and preparation of soil

Soil was collected from an agricultural field in Central Butte, SK, Canada (50.729607 N; -106.422086 W), where the previous year's residues of dry yellow peas, *Pisum sativum* L., remained. The top 15-20 cm of soil was removed to use in the greenhouse experiment. The soil was laid out on trays to air dry for one week and then sieved to 4 mm to remove rocks, large soil aggregates and organic residues such as sticks and leaves. The large aggregates of soil were ground and mixed in with the previously sieved soil. All sieved and ground soil was thoroughly homogenized using a soil mixer. The mixed soil was then sieved to 2 mm and stored until needed.

Air-dried, 2 mm sieved soil was sent to ALS Environmental Laboratory, Saskatoon, Canada for physical characterization and nutrient analysis. Soil texture was determined using the mini-pipet method. Briefly, sodium hexametaphosphate was added to dry sieved (<2 mm) soil to ensure dispersion of soil particles. The homogenized solution was allowed to settle until only clay particles remained in suspension. The clay fraction was measured by removing a sample of the suspension, which was then dried and weighed. The sand fraction was determined through wet sieving the remaining suspension and then weighing the sand in the sieve once dried. The silt fraction was determined using the following equation: % Silt = 100 – (% Sand + % Clay) (USDA NRCS, 2014). Soil organic matter content was determined using the dry-ash method whereby organic matter is removed through combustion at 375 °C for a minimum of 16 hrs (Canadian Soil Survey Committee, 1978). Soil pH and electrical conductivity (EC) were measured using a saturated soil paste. The pH was first measured using a pH meter, and subsequently vacuum filtrated where the resulting extract was measured for EC using a conductivity meter (Janzen, 1993).

Available nitrate and nitrite were measured using methods from Alberta Agriculture (Anonymous, 1988). Briefly, a diluted calcium chloride solution was used to extract nitrate and nitrite from the soil. The nitrate was reduced to nitrite by passing through a copperized cadmium (Cd) column. The nitrite (original plus reduced nitrate) was diazotized with sulfanilamide which was followed by a coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting magenta colored dye was measured colorimetrically at 520 nm. Available sulfate was measured using a weak calcium chloride solution. Sulfate in the subsequent extract was measured using inductively coupled plasma optical emission spectrometry (ICP-OES) (Schoenau and Karamanos, 1993). Plant available phosphorous (P) and potassium (K) were measured using a modified Kelowna solution. Phosphorous was determined colorimetrically at 880 nm from the soil extract, whereas K was measured by flame emission at 770 nm (Qian et al., 1994). Basic salinity and cations were measured in a saturated soil extract. Calcium (Ca), magnesium (Mg), sodium (Na) and K were measured by ICP-OES (Janzen, 1993).

The soil texture was classified as a loam with 50.3 % sand, 40.6 % silt, and 9.1 % clay. The organic matter content of the soil was 3.3 % as determined by loss on ignition at 375 °C. The soil pH was slightly basic at 7.43 and the soil was classified as non-saline based on the EC of 0.74 dS m^{-1}. Available nitrate, sulfate, P and K were 11.7, 5.5, 19.7 and 514 mg kg^{-1}, respectively.

Sodium adsorption ratio was 0.37 while the Ca, P, Mg and Na levels in the soil were 70.1, 59.3, 21.6 and 13.8 mg kg^{-1}, respectively.

4.4.2 Contamination and incubation of soil

The five plant species evaluated in the previous study (Chapter 3) [*Achillea millefolium* L. (common yarrow); *Astragalus canadensis* L. (Canadian milkvetch); *Calamovilfa longifolia* (Hook.) Scribn. (Prairie sandreed); *Koeleria macrantha* (Ledeb.) Schult. (Prairie Junegrass); *Vicia americana* Muhl. ex Willd (American vetch)] were grown in artificially contaminated soil. Soil, sieved to 2 mm, was weighed into pots 7.5 cm in diameter. Each pot was lined with a Ziploc® bag to prevent contaminants from being washed out of the soil. Each pot received 300 g of soil. Contaminant solutions were added to soil using the following: sodium sulfate (Na_2SO_4), sodium chloride (NaCl), potassium sulfate (K_2SO_4), potassium chloride (KCl), cadmium nitrate [$Cd(NO_3)_2$], chromium nitrate [$Cr(NO_3)_2$], copper (II) nitrate [$Cu(NO_3)_2$], nickel (II) nitrate [$Ni(NO_3)_2$]. Salt solutions were made using deionized water, whereas metal solutions were prepared using double deionized water and acid washed glassware. One week prior to planting, 54 mL of contaminated solution was added to pots. Sulfate and chloride were added at 1000 and 5000 mg kg^{-1} of soil; Cd, Cu, and Ni were added at 10 and 20 mg kg^{-1}, and Cr was added at 10 and 50 mg kg^{-1} of soil. Contaminant levels were chosen based on what has been used in other studies of similar species (i.e., flowering plants and grasses) (Nedelkoska and Doran, 2001; Ghosh and Singh, 2005; Li et al., 2016) and results from the previous study (Chapter 3). No fertilizers or amendments were added to soil during the experiment. Soil was incubated for one week to minimize fluctuations of the recovering soil microbial community (Gordon et al., 2008). Pots were incubated in a Conviron growth chamber at the University of Saskatchewan under a day-night schedule of 14 daylight hours and 10 night hours with temperatures fluctuating between 16 °C at night and peaking at 24 °C during the day.

Each treatment had four replicates. Pots were grouped based on plant species, arranged in a randomized design using a random number generator. Pots were rotated daily using plastic trays to eliminate edge biases, and were watered daily with double deionized water to maintain a 50 % field water holding capacity.

One week after contamination, four seeds were planted per pot at a depth of 0.5 cm. Date of seed germination was noted for each pot. If multiple seeds germinated the same day in a single pot, the biggest one was left after thinning. Thinning was done one week post-planting. Pots were

thinned to one seedling per pot. Seedlings grew for 42 d post-emergence. Since contaminant concentrations in the pots may have elicited similar plant responses as that of hotspots, seeds were replanted up to two times when no seeds germinated or seeds germinated and died. Replanting was done at two and four weeks after the initial planting. Pots that had no seed germination 28 d after the most recent planting were removed from rotation and no longer watered.

Plants were harvested 42 d post-emergence. Soil was gently loosened to minimize root breakage, and soil was thoroughly scanned for any broken roots or leaf biomass. The entire plant and resulting broken roots and lost leaves were then washed to remove all remaining visible soil particles using a spray bottle filled with double deionized water. Washed plants were blotted with paper towel to remove excess water. Above and below ground biomass was then divided and weighed. Biomass was placed in a drying oven at 80 °C for 48 hrs to determine dry weight (Su et al., 2005; Cambrollé et al., 2012). Dried biomass was stored in envelopes for future elemental analysis.

4.4.3 Statistical analysis

Statistical analysis was carried out using SAS® 9.4 (SAS Institute Inc., 2016). A one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test were used to compare germination rates and biomass weights (root, shoot, total) between contaminant treatments of the various plant species. All data sets included zeros to average results over the four planted pots. Normality of data was assessed using Shapiro-Wilk's test. Over 90 % of the treatments met normality requirements. If the normality of data was not met, it was analyzed as if it was since it is difficult to ascertain normality with a small sample size.

4.5 Results

4.5.1 Qualitative plant results

Visual differences were observed in soil prior to planting. There was no visual differentiation between the control and metal amended soil; however, salt amended soil had salt crusts appearing on the soil surface during incubation (Fig. 4.1).

Plants grown in the metal amended soil were visually the same as those grown in unamended soil. However, plants grown in salt amended soil, were often stunted and appeared chemically burnt. Multiple seeds germinated in the salt amended soil; however, they often died. Some plant species, notably *A. millefolium* and *A. canadensis*, had many older leaves die and fall

off in all treatments. Multiple leaves, both dead and living, fell off *V. americana* plants, however, all treatments exhibited this, even the control.

Figure 4.1. Salt crust observed on soil amended with Na_2SO_4 prior to seeding.

Astragalus canadensis exhibited decreased lateral root growth in some of the treatments, namely 10 mg kg^{-1} Cu, 50 mg kg^{-1} Cr, and 1000 and 5000 mg kg^{-1} K_2SO_4. Red colored *A. canadensis* roots were seen in 20 mg kg^{-1} Cu and Ni, 50 mg kg^{-1} Cr and 5000 mg kg^{-1} K_2SO_4 (Fig. 4.2).

4.5.2 Emergence

Only emergence from the first round of planting was used to calculate emergence. Seeds that emerged from the second and third rounds of planting were not considered in total emergence. No significant emergence differences were observed in *A. canadensis, A. millefolium, C. longifolia* and *V. americana* compared to the controls in the first round of planting; however, *K. macrantha* exposed to 1000 mg kg^{-1} K_2SO_4 had significantly ($p<0.05$) lower emergence than the control. All 5000 mg kg^{-1} salt treatments and 1000 mg kg^{-1} NaCl completely inhibited *A. millefolium* emergence for the first planting. Similarly, *A. canadensis*, *C. longifolia*, *K. macrantha*, and *V. americana* germination was completely inhibited by salt treatments including chloride and 5000 mg kg^{-1} Na_2SO_4. Emergence of *C. longifolia* and *V. americana* was also inhibited by 5000 mg kg^{-1} K_2SO_4.

Vicia americana seeds exposed to 10 mg kg^{-1} Cu had significantly (p<0.05) higher germination compared to seeds exposed to 1000 mg kg^{-1} Na_2SO_4 (Table 4.1). Photos of plants taken just prior to harvesting can be seen in Appendix B.

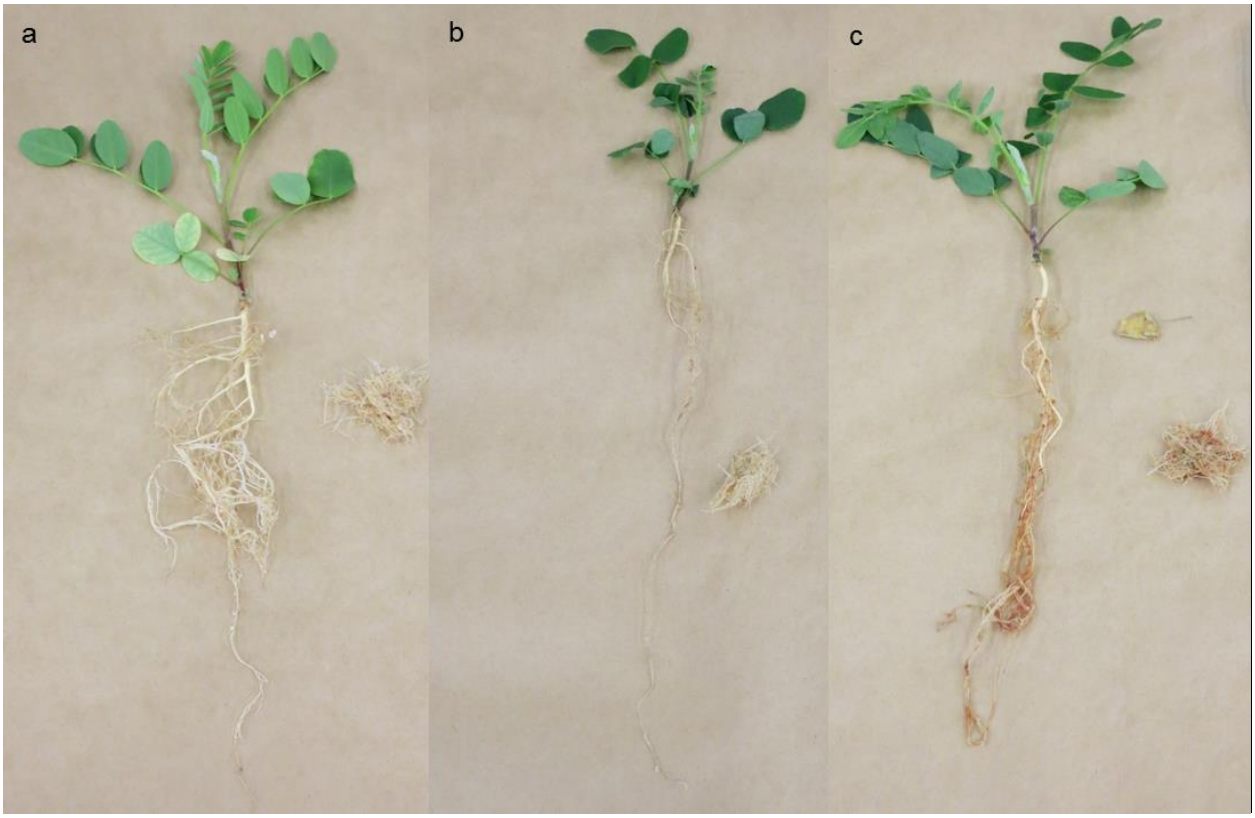

Figure 4.2. Visual differences in roots of *Astragalus canadensis*: (a) control, (b) decreased lateral root growth in 10 mg kg^{-1} Cu, and (c) decreased lateral root growth, and red colored roots in 50 mg kg^{-1} Cr.

4.5.3 Dried root, shoot and total biomass

The root biomass of *A. millefolium* and *V. americana* were significantly (p<0.05) lower in salt treatments as compared to the control, except for root biomass produced in both 1000 mg kg^{-1} potassium and sodium sulfate, which was similar to the control (Table 4.2). The same was observed for *A. canadensis* and *K. macrantha* roots. Conversely, no biomass differences were noted between *C. longifolia* treatments and the control. Biomass of *C. longifolia* roots was significantly (p<0.05) higher in 10 mg kg^{-1} Cu than 5000 mg kg^{-1} K_2SO_4.

Table 4.1: Emergence of Canadian native plant species grown in metal and salt spiked agricultural soil incubated between 16 and 24 °C at 50 % water holding capacity

Treatment	Dose (mg kg^{-1})	Emergence (%) ± SD*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control	0	25.0 ± 20.4 ab	56.2 ± 12.5 a	18.8 ± 23.9 a	62.5 ± 32.2 a	43.8 ± 12.5 abc
KCl	1000	0.0 ± 0.0 b	18.8 ± 12.5 ab	0.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c
	5000	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c
NaCl	1000	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c
	5000	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c
K_2SO_4	1000	31.3 ± 23.9 ab	37.5 ± 25.0 ab	37.5 ± 25.0 a	37.5 ± 14.4 abc	25.0 ± 0 abc
	5000	6.3 ± 12.5 ab	0.0 ± 0.0 b	0.0 ± 0.0 a	6.3 ± 12.5 bc	0.0 ± 0.0 c
Na_2SO_4	1000	0.1 ± 0.1 ab	12.5 ± 14.4 ab	18.8 ± 12.5 a	31.3 ± 23.9 abc	6.3 ± 12.5 bc
	5000	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c
Cd	10	25.0 ± 35.4 ab	50.0 ± 40.8 ab	43.8 ± 37.5 a	43.8 ± 23.9 ab	37.5 ± 32.3 abc
	20	25.0 ± 20.4 ab	56.3 ± 23.9 a	37.5 ± 32.3 a	62.5 ± 14.4 ab	43.8 ± 31.5 abc
Cr	10	62.5 ± 25.0 a	37.5 ± 25.0 ab	18.8 ± 23.9 a	37.5 ± 14.4 abc	56.3 ± 37.5 ab
	50	43.8 ± 51.5 ab	31.3 ± 12.5 ab	37.5 ± 32.3 a	37.5 ± 14.4 abc	50.0 ± 0.0 abc
Cu	10	37.5 ± 14.4 ab	56.3 ± 23.9 a	31.3 ± 23.9 a	56.2 ± 23.9 a	62.5 ± 32.3 a
	20	43.8 ± 23.9 ab	56.3 ± 31.5 a	50.0 ± 20.4 a	43.8 ± 12.5 ab	43.8 ± 12.5 abc
Ni	10	31.3 ± 31.5 ab	43.8 ± 23.9 ab	37.5 ± 32.3 a	56.3 ± 12.5 a	56.3 ± 23.9 ab
	20	37.5 ± 14.4 ab	50.0 ± 35.4 ab	31.3 ± 23.9 a	43.8 ± 23.9 ab	43.8 ± 37.5 abc

*Mean ± standard deviation (SD) where different letters in the same column are significantly different using Tukey's HSD (n=4, Cr 50 n=3, $p<0.05$)

Table 4.2: Dried root biomass of Canadian native plant species grown metal and salt spiked agricultural soil incubated between 16 and 24 °C at 50 % water holding capacity, oven dried at 80 °C for 48 hrs

Treatment	Dose (mg kg^{-1})	Root biomass (g) ± SD*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control	0	0.36 ± 0.03 ab	0.73 ± 0.15 a	0.11 ± 0.10 ab	0.21 ± 0.11 a	0.26 ± 0.03 a
KCl	1000	0.00 ± 0.00 c	0.25 ± 0.19 bcde	0.00 ± 0.00 b	0.01 ± 0.02 cd	0.01 ± 0.01 b
	5000	0.00 ± 0.00 c	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 b
NaCl	1000	0.00 ± 0.00 c	0.20 ± 0.15 de	0.00 ± 0.00 b	0.05 ± 0.10 bcd	0.00 ± 0.00 b
	5000	0.00 ± 0.00 c	0.01 ± 0.02 e	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 b
K_2SO_4	1000	0.27 ± 0.05 ab	0.53 ± 0.15 abcd	0.10 ± 0.03 ab	0.18 ± 0.05 ab	0.19 ± 0.03 a
	5000	0.01 ± 0.01 c	0.22 ± 0.12 cde	0.01 ± 0.03 b	0.04 ± 0.06 bcd	0.01 ± 0.01 b
Na_2SO_4	1000	0.25 ± 0.18 abc	0.61 ± 0.17 abc	0.10 ± 0.09 ab	0.13 ± 0.02 abcd	0.31 ± 0.08 a
	5000	0.00 ± 0.00 c	0.01 ± 0.02 e	0.03 ± 0.05 ab	0.00 ± 0.00 d	0.02 ± 0.02 b
Cd	10	0.21 ± 0.16 bc	0.80 ± 0.17 a	0.06 ± 0.05 ab	0.18 ± 0.03 ab	0.31 ± 0.06 a
	20	0.31 ± 0.04 ab	0.72 ± 0.1 a	0.09 ± 0.06 ab	0.19 ± 0.05 ab	0.33 ± 0.12 a
Cr	10	0.29 ± 0.07 ab	0.74 ± 0.01 a	0.09 ± 0.07 ab	0.23 ± 0.08 a	0.24 ± 0.06 a
	50	0.17 ± 0.12 bc	0.83 ± 0.26 a	0.10 ± 0.03 ab	0.13 ± 0.08 abcd	0.28 ± 0.20 a
Cu	10	0.21 ± 0.06 bc	0.75 ± 0.12 a	0.15 ± 0.06 a	0.21 ± 0.04 a	0.27 ± 0.08 a
	20	0.34 ± 0.09 ab	0.82 ± 0.14 a	0.09 ± 0.04 ab	0.16 ± 0.02 abc	0.29 ± 0.02 a
Ni	10	0.49 ± 0.27 a	0.74 ± 0.21 a	0.07 ± 0.05 ab	0.24 ± 0.10 a	0.24 ± 0.03 a
	20	0.32 ± 0.06 ab	0.64 ± 0.28 ab	0.12 ± 0.04 ab	0.16 ± 0.04 abc	0.27 ± 0.02 a

*Mean ± standard deviation (SD) where different letters in the same column are significantly different using Tukey's HSD (n=4, Cr 50 n=3, $p<0.05$)

Many of the *A. millefolium* salt amended treatments: 1000 and 5000 mg kg^{-1} KCl, 5000 mg kg^{-1} NaCl and 5000 mg kg^{-1} Na_2SO_4, produced significantly ($p<0.05$) less shoot biomass, as compared to the control (Table 4.3). Like *A. millefolium*, *C. longifolia* shoot biomass was significantly lower for chloride and 5000 mg kg^{-1} sulfate treatments. All chloride and 5000 mg kg^{-1} sulfate amended soil planted with *K. macrantha* and *A. canadensis* produced significantly ($p<0.05$) less root biomass than the control. There was no significant difference in *C. longifolia* root biomass in metal amended treatments.

The total biomass of metal amended *A. millefolium* was not significantly different from the control (Table 4.4). However, all of the *A. millefolium* exposed to salts, except for 1000 mg kg^{-1} sulfate, had significantly ($p<0.05$) lower total biomass when compared to the control. Similarly, *A. canadensis, K. macrantha* and *V. americana* showed no difference in total biomass between metal treatments and the control.

Dry weight root and shoot ratios (R:S) were calculated. There was no significant ($p<0.05$) difference in ratio between the control and the treatments of *A. canadensis*, *C. longifolia*, and *V. americana* (Table 4.5). Metal treatments, like biomass, did not significantly affect the R:S. *Achillea millefolium* exhibited significant ($p<0.05$) decreases in the R:S for all salts exceeding 5000 mg kg^{-1}, and 1000 mg kg^{-1} NaCl. All the chloride and 5000 mg kg^{-1} Na_2SO_4 contaminated soil significantly ($p<0.05$) decreased the R:S ratio of *K. macrantha*.

There were no significant differences in root or shoot biomass between metal treatments and the control, for any plant species, at any contamination level. There was a trend that chloride had a higher growth inhibitory effect on plant growth than did sulfate, and that the plants' resistance to chloride in soil was lower than 1000 mg kg^{-1}. Results also suggest that a plant's sulfate limit lies between 1000 and 5000 mg kg^{-1}. Results indicate that there was no difference in plant biomass between the sodium and potassium salts, though it was thought that plants would grow better in potassium since it is a macronutrient.

Table 4.3: Dried shoot biomass of Canadian native plant species grown metal and salt spiked agricultural soil incubated between 16 and 24 °C at 50 % water holding capacity, oven dried at 80 °C for 48 hrs

Treatment	Dose (mg kg^{-1})	Shoot biomass (g) ± SD*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control	0	0.53 ± 0.08 ab	0.45 ± 0.02 abcd	0.19 ± 0.17 abc	0.28 ± 0.17 a	0.37 ± 0.03 a
KCl	1000	0.00 ± 0.00 c	0.20 ± 0.13 ef	0.00 ± 0.00 c	0.02 ± 0.03 c	0.01 ± 0.01 b
	5000	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
NaCl	1000	0.00 ± 0.00 c	0.22 ± 0.15 def	0.00 ± 0.00 c	0.07 ± 0.14 bc	0.00 ± 0.00 b
	5000	0.00 ± 0.00 c	0.01 ± 0.03 f	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
K_2SO_4	1000	0.37 ± 0.08 abc	0.40 ± 0.10 bcde	0.20 ± 0.04 abc	0.27 ± 0.07 a	0.32 ± 0.06 a
	5000	0.01 ± 0.01 c	0.27 ± 0.11 cde	0.02 ± 0.04 bc	0.06 ± 0.08 bc	0.01 ± 0.02 b
Na_2SO_4	1000	0.37 ± 0.27 abc	0.47 ± 0.06 abc	0.19 ± 0.14 abc	0.23 ± 0.05 ab	0.30 ± 0.04 a
	5000	0.00 ± 0.00 c	0.01 ± 0.02 f	0.04 ± 0.08 bc	0.00 ± 0.00 c	0.02 ± 0.03 b
Cd	10	0.36 ± 0.24 bc	0.59 ± 0.10 ab	0.14 ± 0.10 abc	0.36 ± 0.13 a	0.41 ± 0.07 a
	20	0.42 ± 0.02 ab	0.59 ± 0.04 ab	0.13 ± 0.10 abc	0.28 ± 0.05 a	0.37 ± 0.05 a
Cr	10	0.41 ± 0.08 ab	0.55 ± 0.08 ab	0.18 ± 0.13 abc	0.33 ± 0.06 a	0.38 ± 0.07 a
	50	0.28 ± 0.19 bc	0.66 ± 0.04 a	0.31 ± 0.13 a	0.18 ± 0.08 abc	0.38 ± 0.06 a
Cu	10	0.39 ± 0.10 ab	0.61 ± 0.04 ab	0.26 ± 0.12 ab	0.35 ± 0.06 a	0.34 ± 0.05 a
	20	0.57 ± 0.02 ab	0.61 ± 0.08 ab	0.19 ± 0.11 abc	0.28 ± 0.06 a	0.41 ± 0.07 a
Ni	10	0.74 ± 0.40 a	0.51 ± 0.16 abc	0.12 ± 0.09 abc	0.35 ± 0.08 a	0.35 ± 0.07 a
	20	0.48 ± 0.05 ab	0.49 ± 0.18 abc	0.21 ± 0.05 abc	0.30 ± 0.05 a	0.40 ± 0.05 a

*Mean ± standard deviation (SD) where different letters in the same column are significantly different using Tukey's HSD (n=4, Cr 50 n=3, $p<0.05$)

Table 4.4: Dried total biomass of Canadian native plant species grown metal and salt spiked agricultural soil incubated between 16 and 24 °C at 50 % water holding capacity, oven dried at 80 °C for 48 hrs

Treatment	Dose (mg kg^{-1})	Total biomass (g) ± SD*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control	0	0.89 ± 0.10 ab	1.18 ± 0.13 a	0.30 ± 0.28 ab	0.49 ± 0.27 a	0.63 ± 0.02 ab
KCl	1000	0.00 ± 0.00 d	0.45 ± 0.32 bc	0.00 ± 0.00 b	0.02 ± 0.05 d	0.02 ± 0.02 c
	5000	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 c
NaCl	1000	0.00 ± 0.00 d	0.42 ± 0.29 bc	0.00 ± 0.00 b	0.12 ± 0.24 bcd	0.00 ± 0.00 c
	5000	0.00 ± 0.00 d	0.02 ± 0.04 c	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 c
K_2SO_4	1000	0.64 ± 0.12 abc	0.93 ± 0.23 ab	0.30 ± 0.06 ab	0.45 ± 0.10 ab	0.51 ± 0.08 b
	5000	0.02 ± 0.02 cd	0.49 ± 0.23 bc	0.03 ± 0.06 b	0.10 ± 0.14 cd	0.02 ± 0.03 c
Na_2SO_4	1000	0.62 ± 0.45 abcd	1.09 ± 0.22 a	0.29 ± 0.23 ab	0.36 ± 0.06 abc	0.61 ± 0.07 ab
	5000	0.00 ± 0.00 d	0.02 ± 0.04 c	0.07 ± 0.13 ab	0.00 ± 0.00 d	0.03 ± 0.05 c
Cd	10	0.57 ± 0.40 bcd	1.38 ± 0.16 a	0.20 ± 0.15 ab	0.54 ± 0.14 a	0.72 ± 0.08 a
	20	0.73 ± 0.06 ab	1.30 ± 0.13 a	0.22 ± 0.15 ab	0.47 ± 0.09 a	0.70 ± 0.08 ab
Cr	10	0.70 ± 0.14 ab	1.29 ± 0.08 a	0.27 ± 0.20 ab	0.56 ± 0.14 a	0.62 ± 0.12 ab
	50	0.45 ± 0.30 bcd	1.48 ± 0.25 a	0.41 ± 0.12 a	0.32 ± 0.16 abcd	0.65 ± 0.25 ab
Cu	10	0.60 ± 0.13 bcd	1.36 ± 0.09 a	0.40 ± 0.18 a	0.56 ± 0.05 a	0.61 ± 0.07 ab
	20	0.90 ± 0.11 ab	1.43 ± 0.21 a	0.28 ± 0.14 ab	0.43 ± 0.07 ab	0.70 ± 0.08 ab
Ni	10	1.23 ± 0.68 a	1.25 ± 0.37 a	0.18 ± 0.14 ab	0.60 ± 0.17 a	0.59 ± 0.08 ab
	20	0.80 ± 0.06 ab	1.13 ± 0.45 a	0.33 ± 0.09 ab	0.45 ± 0.08 a	0.67 ± 0.04 ab

*Mean ± standard deviation (SD) where different letters in the same column are significantly different using Tukey's HSD (n=4, Cr 50 n=3, $p<0.05$)

Table 4.5: Root:shoot ratios of dried biomass of Canadian native plant species grown metal and salt spiked agricultural soil incubated between 16 and 24 °C at 50 % water holding capacity, oven dried at 80 °C for 48 hrs

Treatment	Dose (mg kg^{-1})	Means ± SD (g)*				
		Astragalus canadensis	*Achillea millefolium*	*Calamovilfa longifolia*	*Koeleria macrantha*	*Vicia americana*
Control		0.69 ± 0.07 ab	1.62 ± 0.43 a	0.41 ± 0.28 abc	0.75 ± 0.16 a	0.70 ± 0.10 ab
KCl	1000	0.00 ± 0.00 b	1.22 ± 0.24 ab	0.00 ± 0.00 c	0.13 ± 0.25 cd	0.42 ± 0.50 ab
	5000	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
NaCl	1000	0.00 ± 0.00 b	0.71 ± 0.51 bc	0.00 ± 0.00 c	0.19 ± 0.37 bcd	0.00 ± 0.00 b
	5000	0.00 ± 0.00 b	0.15 ± 0.30 c	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
K_2SO_4	1000	0.75 ± 0.08 ab	1.35 ± 0.31 ab	0.47 ± 0.11 abc	0.65 ± 0.12 a	0.58 ± 0.03 ab
	5000	0.63 ± 0.95 ab	0.76 ± 0.18 bc	0.18 ± 0.36 bc	0.47 ± 0.33 abc	0.38 ± 0.48 ab
Na_2SO_4	1000	0.51 ± 0.34 ab	1.28 ± 0.22 ab	0.36 ± 0.27 abc	0.60 ± 0.11 ab	1.07 ± 0.33 a
	5000	0.00 ± 0.00 b	0.25 ± 0.50 c	0.16 ± 0.31 bc	0.00 ± 0.00 d	0.46 ± 0.53 ab
Cd	10	0.43 ± 0.30 ab	1.41 ± 0.45 ab	0.33 ± 0.25 abc	0.54 ± 0.15 abc	0.78 ± 0.19 a
	20	0.72 ± 0.06 a	1.22 ± 0.15 ab	0.83 ± 0.45 abc	0.65 ± 0.09 a	0.95 ± 0.50 a
Cr	10	0.71 ± 0.08 ab	1.37 ± 0.20 ab	0.37 ± 0.27 abc	0.70 ± 0.15 a	0.63 ± 0.07 ab
	50	0.48 ± 0.35 ab	1.27 ± 0.43 ab	0.38 ± 0.16 abc	0.69 ± 0.12 a	0.70 ± 0.41 ab
Cu	10	0.57 ± 0.15 ab	1.25 ± 0.26 ab	0.59 ± 0.04 ab	0.63 ± 0.25 ab	0.83 ± 0.30 a
	20	0.59 ± 0.15 ab	1.34 ± 0.14 ab	0.53 ± 0.11 abc	0.57 ± 0.07 abc	0.72 ± 0.11 ab
Ni	10	0.66 ± 0.05 ab	1.45 ± 0.06 ab	0.40 ± 0.28 abc	0.67 ± 0.14 a	0.69 ± 0.18 ab
	20	0.68 ± 0.18 ab	1.30 ± 0.27 ab	0.59 ± 0.04 ab	0.53 ± 0.11 abc	0.69 ± 0.14 ab

*Mean ± standard deviation (SD) where different letters in the same row are significantly different (n=4, Cr 50 n=3, $p<0.05$)

4.6 Discussion

4.6.1 Plant germination

Seed germination of all the plant species was unaffected by the metals (10-50 mg kg^{-1}). Some studies report metal contaminated soil can increase seed germination (Schroeder et al., 2005; Banks et al., 2006), whereas others observed germination inhibition (An, 2004). However the study by An (2004) tested Cd at rates 32 times greater, 640 mg Cd kg^{-1}, than what was assessed in my study. It was suggested that seed germination was not a good measure of soil metal toxicity as seeds are quite resistant (An, 2004). The metal concentrations used in my study were chosen based on germination results from a semi-solid water agar assay (Chapter 3); therefore, it is possible that the seeds of the plants tested are more resilient to metals when they are grown in a soil medium. The resiliency may stem from some of the metal not being bioavailable (i.e., adsorbed to organic matter, or metal speciation), or seeds overcame environmental stress with the aid of nutrients present in soil, absent from the agar study.

Grass species, *Poa pratensis* L., did not germinate well in the presence of Ni, arsenic (As) or Cu, and only had a 50 % survival post-emergence, likely due to the high concentrations of metals (200-17 000 mg kg^{-1}); however when used in a grass mixture, *P. pratensis*' germination doubled and survival increased to 100 % (Zacarías et al., 2012). Since the plants in my study were evaluated on their own, it would be interesting to evaluate them when they are part of a mix of species as their resistance to contaminants may increase. Most studies evaluate plant species individually, which is needed to assess their individual resistance and growth capacity; however, seed mixes also require evaluation since phytoremediation mimics the surrounding environment. Plants have varying resistance levels, symbioses and growth characteristics, therefore a variety of seeds planted together would provide a buffer-like capacity (i.e., for weeds and nutrients), and improve growth in a heterogeneously planted environment, compared to a monoculture (Lawson et al., 2015; Felton et al., 2016; Baraibar et al., 2018); however, the opposite has also been observed (Lee et al., 2007).

Many of the salt treatments inhibited germination, sometimes completely, for multiple plant species. The 1000 mg kg^{-1} chloride treatments which did not affect germination in the previous study (Chapter 3) inhibited all five tested species. On the contrary, Bai et al. (2014) found that germination within a seedbank increased when the soil salinity was less than 2000 mg kg^{-1} of NaCl, as compared to the control; however, over 2000 mg kg^{-1} germination was inhibited and dropped

below the control. One reason for this damping off effect is due to reduced water uptake leading to late or inhibited germination (Uhvits, 1946; Katembe et al., 1998).

4.6.2 Plant biomass and R:S

Atriplex nummularia Lindl. produced higher overall biomass when seeds were planted more densely while under Na^+ and Cl^- stress, improving soil conditions by creating macropores, increasing water infiltration and soil biological activity (Silva et al., 2016). None of the five tested species in my study grew well, if at all, in the chloride (KCl, NaCl) contaminated soils. Since all of the species grew well in the metal contaminated soil, it is worth investigating whether a denser seeding, more similar to what would occur in the field, would improve biomass production. Because the oil sands tailings ponds are affected by both metals and salts, it is imperative that phytoremediation species chosen are resistant to the contaminants present.

According to one review (Prasad and Freitas, 2003), plant families dominating phytoremediation and metal hyperaccumulation include Asteraceae, Fabaceae and Poaceae. All of the species tested in my study are from the aforementioned hyperaccumulating families. *Phaseolus vulgaris* L., a member of the Fabaceae family, produced low biomass in Cd contaminated soil making it an ineffective Cd accumulator. However, in the presence of Cr, Fe, Ni, Pb and Zn all showed a positive correlation between the harvested biomass and the metals removed through biomass (Ciura et al., 2005). A potential salt phytoremediator, *Glycyrrhiza glabra* L., also from the Fabaceae family, has the ability to rehabilitate sodic soils to where less sodic tolerant crops can be grown (Dagar et al., 2015). There was no comprehensive list found for salt accumulating species; however, since metal hyperaccumulating species tend to be grouped by families it is possible that the same holds true for salts and remains to be explored.

During plant harvesting, it was noted that *A. canadensis* exhibited decreased lateral root growth for some of the treatments, specifically for 10 mg kg^{-1} Cu, 50 mg kg^{-1} Cr, and 1000 and 5000 mg kg^{-1} K_2SO_4. This inhibition of natural root structure can impede water and nutrient uptake. Other researchers have observed diminished lateral root growth in *A. canadensis* when exposed to elevated levels of selenium (Se) (Goodson et al., 2003). The inhibition of lateral root growth in my study may suggest root avoidance which can indicate a non-accumulating species (Hartikainen et al., 2001). Though there was decreased lateral root growth found in *A. canadensis* for two metal treatments (10 mg kg^{-1} Cu, 50 mg kg^{-1} Cr,), there was no significant decrease in biomass when compared to the control plants. It is possible that the energy normally used for lateral root growth

was reallocated to the tap roots (Aguirrezabal et al., 1994). Other species subject to Cr affected soils have demonstrated decreases in root growth; however, root structure was not mentioned (DelBubba et al., 2013).

Root and shoot dry weights of *Ricinus communis* L. decreased when grown in soil amended with approximately 20 mg kg^{-1} Cd, however there was no visible morphological differences when compared to the control (Bauddh et al., 2016). Root, shoot, and total biomasses of the plant species tested in my experiment did not decrease when exposed to Cd, or to other metals. There were also no visible morphological changes which could indicate a higher plant tolerance or that the metals were bio-unavailable (D'Amore et al., 2005). Pedogenic metal species are generally less bioavailable that those coming from anthropogenic sources (Kuo et al., 1983; Kaasalainen and Yli-Halla, 2003), and adsorption-desorption equilibria must also be considered (Pokrovsky et al., 2012).

Decreases in total biomass were not observed in any of the plants in the metal treatments tested. However, numerous other studies investigating similar plants detected metal sensitivity at contaminants levels lower than those used in my study (Hechmi et al., 2014; González et al., 2015). *Phragmites australis*' (Cav.) Trin. ex Steud. biomass significantly decreased from approximately 25 to less than 5 g of biomass per pot when exposed to 5 mg kg^{-1} of Cd (Hechmi et al., 2014). However, when *P. australis* was grown on substrates of varying Cd, Cu, Ni, Pb and Zn (Cd ranging from trace-34 µg g^{-1}, Cu from 7.4-104 µg g^{-1}, and Ni from 43-48 µg g^{-1}) the resulting biomass was similar in all treatments (Ye et al., 1998). Since the metals were added as nitrates and the soil used in my study was low in nitrogen (11.7 mg kg^{-1}), the additional nitrate in the metal solution may have buffered the stress of the metals minimizing biomass inhibition. The addition of nitrogen to plants has been shown to be beneficial to plants under stress such as contamination (Giansoldati et al., 2012).

Turfgrass species, *Poa pratensis* and *Festuca arundinacea* Schreb., did not have lower biomass when exposed to Cd concentrations of 40 mg kg^{-1} (Xu and Wang, 2014). This is consistent with what was observed for the species screened in my study. Soil Cr levels did reach and surpass the 40 mg kg^{-1} level and showed no negative effects on plant growth. *Lemna minor* L. showed significant decreases in frond lengths when grown in Cr contaminated solution at concentrations as low as 0.5 mg kg^{-1} of potassium chromate (Reale et al., 2016) and *Nicotiana langsdorffii* Weinm. had significantly less biomass when it was grown in 50 mg kg^{-1} when compared to its Cr-free

control (DelBubba et al., 2013). This range of tolerance is the reason why each plant species needs to be evaluated individually for contaminant sensitivity.

Switchgrass was evaluated for heavy metal tolerance, specifically Cd, Cr and Zn, and switchgrass biomass increased in the presence of 10 µM (~0.52 mg kg^{-1}) of Cr and Zn, a phenomenon known as hormesis (Chen et al., 2012). Hormesis is defined as plant growth enhancement at low contaminant concentrations whereas higher doses inhibit growth (Southam and Ehrlich, 1943). Hormesis was also observed in *Coronopus didymus* L. grown in Cd amended soil, up to 200 mg kg^{-1} (Sidhu et al., 2017). In my study, hormesis was not observed for any of the salt or metal treatments which may be due to the plant species or the higher levels tested for each of the metals than those at which hormesis was observed for Chen et al. (2012). However, growth inhibition was not observed either which indicates that the levels of metals tested may be between concentrations of hormesis and growth inhibition. Schwertfeger and Hendershot (2013) raise an alternative theory where the increase in plant growth at low contaminant levels may not be hormesis, but rather an increase in nutrients that were solubilized with the addition of the contaminant. Additional analysis is required to accurately determine what is occurring.

Achillea millefolium is native to Europe but can be found throughout North America (Khela, 2012). Since the metal concentrations tested had no measured effect on *A. millefolium* it would be interesting to see if there was a varietal effect between North American and European varieties. Varietal effects were apparent when turnips were evaluated for Cd accumulation; out of 18 varieties, three showed hyperaccumulator potential (Li et al., 2016). Some plant varieties may have developed coping strategies for dealing with contaminant stress such as vacuole storage or cell wall binding (Ernst et al., 1992; Hall, 2002; Windham et al., 2003), while others may be more adept at growing in drier or nutrient deficient soil.

Known hyperaccumulator, *Pteris vittata*, was evaluated for Cr tolerance and showed a decrease in fresh shoot biomass at 50 mg kg^{-1} Cr, however, tissue concentrations of Cr increased (Su et al., 2005). The authors suggest that even though no visible toxic effects were present at higher Cr concentrations, a decrease in water content of shoots can be a good early indicator of plant stress or toxicity (Su et al., 2005). Similarly, my study also assessed plant growth in 50 mg kg^{-1} Cr and noticed no visible differences between the treatment and control plants. However, the plants' water content was not monitored during the study and, if measured, may have indicated stress or toxicity.

Understanding root biomass dynamics is important for understanding carbon capture and storage in the ecosystem (Cairns et al., 1997). By looking at the R:S there is a better understanding of the dynamics between the root and shoot biomasses. One downside of examining R:S is that the ground is at an arbitrary position (Korner, 1994) and some roots appear aboveground (Jenik, 1971). Depending on vegetation type, there can be a wide range for the R:S (Mokany et al., 2006). Ranges for grasslands and shrublands are 0.34-26.03 (Mokany et al., 2006). The R:S of the current study came in on the lower end of the scale and below. A few explanations for the low R:S include: pots that produced no biomass (i.e., seeds did not germinate or died) were included in the mean calculation lowering the mean and, therefore, the ratio; plants were growing in contaminated soil and were, therefore, stressed; plants were not grown to maturity and, therefore, may have stored energy for plant reproduction (Harper and Ogden, 1970).

While germination of the five species assessed in this study were comparable in many cases, *A. millefolium* had the highest biomass in the majority of the metal and salt treatments. The other species surveyed also did well in many aspects, however *A. millefolium*'s performance stood out. Biomass production and resistance to multiple contaminants are important characteristics of phytoremediation species; therefore, *A. millefolium*'s phytoremediation abilities demand further investigation, specifically its accumulation ability.

4.7 Conclusion

The present study evaluated various Canadian native reclamation approved plant species for their resistance to various salts and metals. The purpose of this study was to compare germination, and above and belowground biomass production of plants grown in metal and salt contaminated soils. The metal treatments did not significantly affect the amount of plant biomass produced nor did it greatly impact seed germination. The salt treatments decreased both above and belowground biomass and had very low germination rates. Results indicate that the levels of salt tested were too high for the plants to adequately germinate even though all seed species germinated well in the 1000 mg kg^{-1} salt treatments of the semi-solid agar study (Chapter 3). Additionally, more screening is required for reclamation approved plants species since plant resistance varies species to species, and variety to variety.

Future directions include elemental analysis of the harvested plant tissue to determine contaminant uptake and storage in the plant biomass. If a plant species demonstrates an ability to accumulate they require further testing in contaminant mixtures to determine interaction effects.

Evaluations of endophytic inoculation as a measure of increasing germination and biomass, and overcoming additive effects of multiple soil contaminants remains to be explored. Kamran et al. (2016) found that Ni uptake increased when *Eruca sativa* Mill. was inoculated with *Pseudomonas putida*. Field trials are also needed to explore the interactions of plant, water and contaminants under fluctuating environmental pressures.

5.0 GENERAL CONCLUSIONS

The vast oil sand tailings ponds are a negative legacy of the bitumen mining process. The settled solids are contaminated with concentrated impurities including metals and salts. Current practices of reclaiming the large area of disturbed land is in progress; however, no remediation work has been done thus far. Remediating what remains of the tailings ponds would go one step further of returning the land to its once productive state. The roots of current reclamation plants may penetrate the >1.0 m layer of overburden (Perry, 1989; Crow, 2005) thereby exposing them to the contaminants below which, over time, will release the once-sealed contaminants into the surrounding area.

Phytoremediation of metals and salts has been greatly studied around the world; however, little information is available on the Canadian native species used in the reclamation of Alberta's oil sands. Numerous species have been suggested as good candidates to reclaim the disturbed area of the tailings ponds; however, many of these plants have not been tested for their remediation capacity or are unavailable commercially, making it difficult to make phytoremediation recommendations. Elevated levels of metals and salts can cause toxicity in plants, cause imbalances in nutrient uptake and impede plant function. Based on seed availability and ease of germination, this study assessed five species recommended for oil sand reclamation in Alberta for their ability to germinate and grow when exposed to elevated levels of metals and salts found in oil sand tailings ponds.

The first objective of this study was to compare germination rates and seedling growth of reclamation approved plant species when exposed to cadmium, chromium, copper, nickel, chloride and sulfate. Germination rates of seeds exposed to metals were largely unaffected; however, cadmium and copper did inhibit *Koeleria macrantha* germination as concentrations increased. In contrast, seeds exposed to salts had significantly lower germination rates than those exposed to metals, and many did not germinate in the salt amended medium exceeding 1000 mg kg^{-1}. Roots of seedlings were more sensitive than shoots and this was consistent throughout contaminants assessed. The concentrations tested in both studies exceeded the salt and metal concentrations of what was found in tailings pond water. The five plant species did not exhibit germination or

biomass inhibition when exposed to metals, and they were generally resistant to sulfate at 1000 mg kg^{-1}. Plants showed the lowest resistance to chloride salts. Given this information and information gleaned from other studies, the plants evaluated in this thesis are recommended for potential use in the reclamation of the tailings sands- keeping in mind that other contaminants (i.e., naphthenic acids) are also present and may inhibit plant growth.

Since all five of the plant species were able to germinate in most of the metal concentrations, and in some of the salt concentrations, these species were also assessed in a pot experiment involving artificially contaminated soil. The second objective of this study was to measure and compare differences in aboveground, belowground and total biomass of plants exposed to various concentrations of metals and salts in soils. Germination, again, was unaffected by the metals present in the soil, as was biomass. However, decreased germination in salt, especially chloride, amended soil was observed across all plant species. Due to germination inhibition or to stunting, only plants grown in salt amended soils had significantly lower biomass as compared to plants grown in the control or metal amended soils.

There were a few limitations of the work presented in this thesis. Only a few of the many species suggested for use in Alberta's soil reclamation were available commercially, and seed dormancy was difficult to break for some species, further limiting the species pool. Additionally, plant species were evaluated separately which is not what is typically seen in the field, nor are contaminants present in isolation. However, contaminant isolation was required as a first step to evaluate each species resistance to the metals and salts.

In conclusion, *Achillea millefolium* had the overall highest biomass out of all of the plant species, and was able to germinate better than other species in the chloride amended soils. In the literature, *A. millefolium* has been used as an indicator of soil and air pollution, and has been observed invading newly disturbed areas. Therefore, *A. millefolium* is being recommended for further study in oil sand tailings pond remediation. A biomass acid digestion should be completed for the biomass collected in the greenhouse study to better determine the species phytoremediation capacities.

5.1 Future Directions

The studies presented in this thesis were a first step of exploring the potential of Canadian native plant species for phytoremediation of the oil sand tailings ponds. Only five species were assessed due to limited seed availability and difficulty breaking seed dormancy. Evaluating metal

and salt uptake is a logical next step for the plant species evaluated in this thesis, along with characterizing their root associated microbiome. Root associated bacteria have been known to buffer plant stress and aid in the uptake of metals (Ma et al., 2011; Glick, 2012). Some studies have already explored using mycorrhizal colonization to buffer saline conditions of grass species, including *Calamovilfa longifolia* (Tsang, 1997). A new fungal species discovered in Indian soils, *Piriformospora indica*, has been shown to reprogram how barley responds to various environmental stressors, including salt stress (Waller et al., 2005). Recognizing that barley was widely used in reclamation this would be a great place to begin examining the influence of bacterial and fungal species on plant species used in reclamation and remediation in relation to contaminant resilience. *Achillea millefolium* and *Vicia americana* have also been observed invading new industrially disturbed areas near Oster and Tawayik Lake (Arychuk, 2001). Other future experiments can include identifying sites in the field to evaluate single vs. mixed species and how salt and metal uptake is affected. Assessing contaminant mixtures is important since many contaminated sites are affected by multiple contaminants, specifically mixtures found at oil sand tailings sites. With salts and metals mixed together, it is common for metals to be neutralized by the salts, essentially making them biologically unavailable. Further testing is required to determine metal bioavailability. With more information on Canadian reclamation species we can play to their strengths to get the best possible outcome when looking to phytoremediate the oil sand tailings ponds of Alberta, Canada.

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APPENDIX A

Germination and mold contamination results of the sterilized vs. non-sterilized seed trial on solid and semi-solid water agar (Chapter 3).

Table A.1: Total germination of select Canadian native plant species grown for 7 d at 23 °C in the dark on semi-solid and solid water agar comparing surface sterilized and non-sterilized seeds

	Germination ± SD (%)*			
	Non-sterilized		Sterilized	
Plant species	Semi-solid	Solid	Semi-solid	Solid
Bromus ciliatus	10.0 ± 10.0	3.3 ± 5.8	6.7 ± 5.8	3.3 ± 5.8
Calamovilfa longifolia	53.3 ± 11.5	56.7 ± 20.8	70.0 ± 10.0	63.3 ± 15.3
Deschampsia caespitosa	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 11.5	3.3 ± 5.8
Elymus canadensis	16.7 ± 5.8	26.7 ± 5.8	23.3 ± 5.8	13.3 ± 5.8
Elymus innovatus	0.0 ± 0.0	10.0 ± 10.0	0.0 ± 0.0	3.3 ± 5.8
Festuca campestris	23.3 ± 15.3	20.0 ± 20.0	16.7 ± 20.8	13.3 ± 11.5
Koeleria macrantha	50.0 ± 26.5	50.0 ± 20.0	83.3 ± 5.8	73.3 ± 23.1
Sporobolus cryptandrus	6.7 ± 5.8	6.7 ± 5.8	10.0 ± 17.3	3.3 ± 5.8
Vicia americana	53.3 ± 30.6	63.3 ± 15.3	56.7 ± 20.8	43.3 ± 5.8

*Mean ± standard deviation (SD)

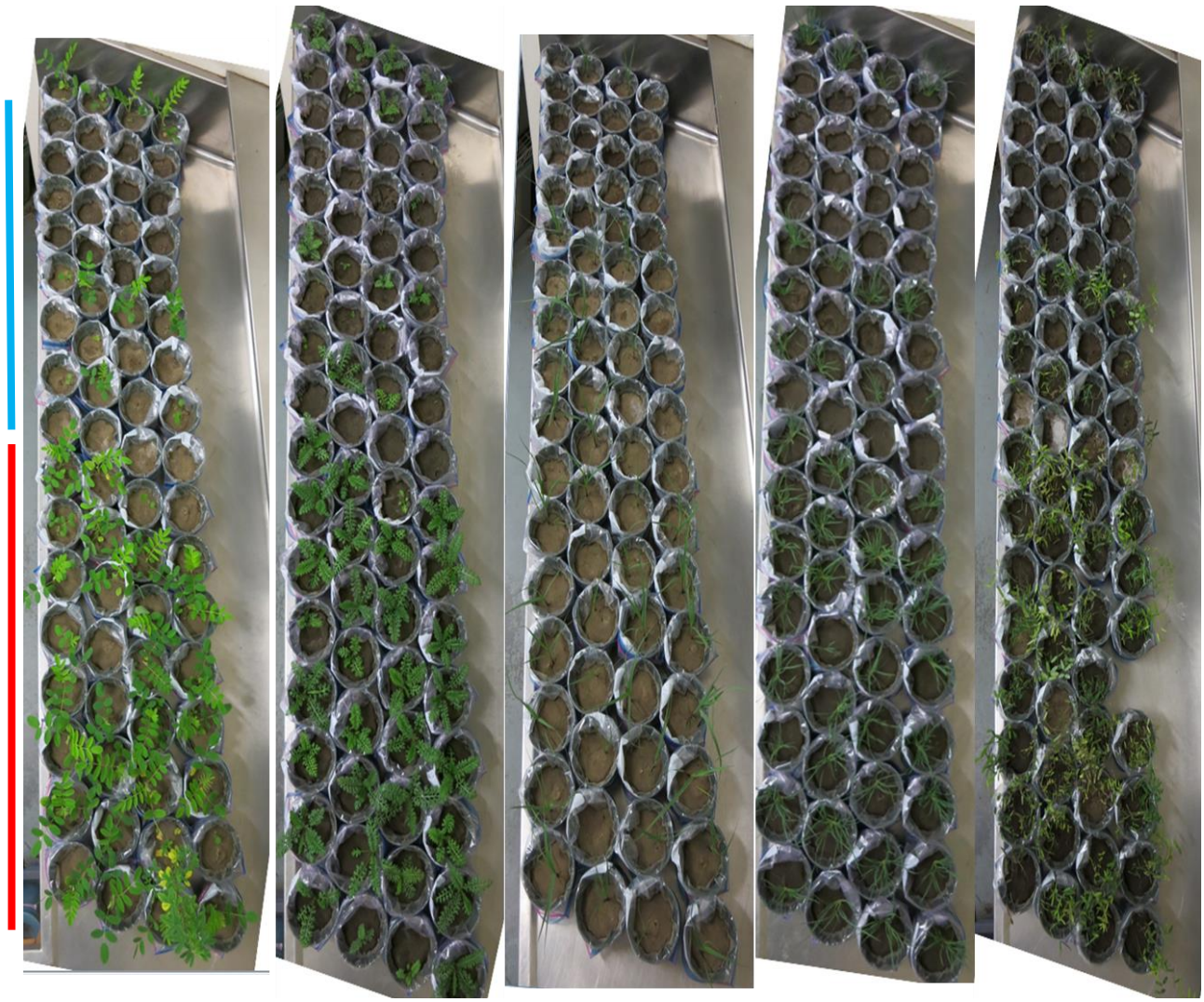
Table A.2: Mold contamination of select Canadian native plant species grown for 7 d at 23 °C in the dark on semi-solid and solid water agar comparing surface sterilized and non-sterilized seeds

	Moldy seeds*			
	Non-sterilized		Sterilized	
Plant species	Semi-solid	Solid	Semi-solid	Solid
Bromus ciliatus	0.0	0.0	0.0	0.0
Calamovilfa longifolia	20.0	Gen 2	0.0	0.0
Deschampsia caespitosa	0.0	0.0	0.0	0.0
Elymus canadensis	13.3	16.7	10.0	6.7
Elymus innovatus	0.0	10.0	0.0	0.0
Festuca campestris	Gen 3	0.0	0.0	Gen 1
Koeleria macrantha	Gen 3	26.7	0.0	0.0
Sporobolus cryptandrus	0.0	0.0	0.0	0.0
Vicia americana	3.3	Gen 1	3.3	0.0

*Percentages refer to the total number of seeds (n_T= 30) per treatment that presented with mold; Gen # refers to general mold on the plate with the number referring to the number of replicates affected (n=3)

APPENDIX B

Photographs of the metal and salt contaminated soil greenhouse experiment (Chapter 4).



A. canadensis *A. millefolium* *C. longifolia* *K. macrantha* *V. americana*

Figure B.1: Plant growth of Canadian native plant species grown in metal and salt contaminated agricultural soil right before harvest. The top row of each column of pots is the Control, the next 8 rows are contaminated with salts, and the final 8 are contaminated with metals.

Figure B.2: An example of *A. canadensis* after harvesting and washing. Note presence of nodules.

Figure B.3: An example of *A. millefolium* after harvesting and washing. Note the spreading of rhizomes.